We as Food and Feeders

Prey of Microbes

A Lion’s Share?

Becoming Food at Death

As we are with respect to our body a physical part of the creation, we represent of course a source of food for other organisms. Since we have experienced a spectacular population growth over the last 10,000 years or so, other organisms will not overlook the naked ape as food. Psychologically, we have lost the impression of representing an integral part of the food chain. However, there are a number of ways for us to become food for other organisms. If our body is buried after death, a sequence of detrivores take care of it. People throughout time were aware of that, and the paintings of the late medieval time in Europe frightened their observers with the specter of decomposing human bodies. In the western hemisphere, we have learned to blot out the idea that death is part of our life as for any other organism and we leave this impression to the specialists of forensic medicine. On the other hand we have got used to the idea that we are the top predators in the biosphere, that we have lost the memory that we can fall victim to predators, large and small.

Attack by Carnivores

Large carnivores still take their toll. In Tanzania lions (Figure 7.1) killed more than 560 people over the last 15 years, and the trend is increasing (Packer et al. 2005). Lions pull people out of bed, attack nursing mothers, and catch playing children. A typical scenario is an attack on a farmer sleeping in a makeshift hut on his fields to protect the crops from nocturnal raids by bush pigs. The rising trend is easily explained: humans intrude into areas where lions live and since they eliminate the natural prey of these large carnivores (kudu, zebra, hartebeest, and the like), lions take humans as a surrogate prey. Similar cases could be told for the Asian tiger, but overall only a negligible part of the world population becomes prey to carnivores.
The tiger (*Leo tigris*, *top*) and the lion (*Leo leo*, *bottom*) are the largest representatives of the cat family (Felidae). The tiger probably evolved in Northern Europe, but moved from there to Siberia and Asia. Individual tigers are known as man-eaters, but normally tigers hunt deer and wild hog. The lion (*Leo leo, bottom*), the king of the animals in the folklore, hunts many animals, ranging in size from insects to giraffes, but its preferred prey is antelope-sized. An old lion might become a man-eater when unable to hunt quicker prey.
Prey of Microbes

So where do we become food ourselves? In affluent societies, more people die of cancer, cardiovascular diseases, or simply of old age than of infectious diseases. This was not always so; just 100 years ago, infectious diseases were a major killer and this remained so in many parts of the world, which we euphemistically call developing countries. If you look at infectious diseases with the eyes of a biologist, you see microorganisms, which make a living from us. In benign cases—and this is the majority—they feed on us without doing too much harm to us. In more serious cases they literally eat us up. It was said that humans fight with insects for supremacy on the globe and it is not yet decided who will win the war. The battle with mosquitoes will be illustrated. Personally, I would not like to bet on either of these two combatants. My personal bet is different: in the beginning of time, microbes were the rulers, and most likely, they will be the dominant form of life at the end of time. Whether mammals will survive the second half of the evolutionary path, which is still ahead of us (if we accept the life expectancy of our sun given by astrophysicists), remains doubtful to me, while bacteria will certainly survive. As this question is idle speculation, I will now explore with you how we become substrate for the growth of microorganisms.

The Haunted Hunter...

Simian Foamy Virus

In a previous section, it was reported that the decline of fish in the local West African market motivated people to search for alternative protein sources in the nearby bush. This bushmeat hunting not only had negative consequences on the population size of animal wildlife, but might be the basis of one of the largest medical challenges currently facing humankind. A recent study in the *Lancet* illustrates this point (Wolfe et al. 2004). Epidemiologists asked 1,800 people from nine rural villages in Cameroon in equatorial Africa; 61% reported direct exposure to primate blood primarily through hunting and butchery. Notably, 16% of the exposed people showed serological evidence of a past infection with simian foamy virus. In 1% the viral exposure was confirmed by a more rigorous test (western blot) and viral RNA was detected in the subjects. As foamy viruses are largely species specific, the sequence analysis suggested three independent cross-species infections. When these sequences were projected on a large number of simian foamy viruses, transmission to humans from three different primates could be identified by phylogenetic analysis (*Cercopithecus*, *Mandrillus*, and *Gorilla*). All the virus-positive persons confirmed contact with animals either through butchery and eating of monkey, chimpanzee or gorilla hunted by guns, bows, or wires or through a pet monkey. Simian foamy viruses do not cause a disease in the natural host nor showed any of the virus-positive human signs of disease in this or previous studies with zoo workers who experienced simian foamy virus infections. Although the simian-to-human transmission is relatively high (about 2%), no human-to-human transmission was yet reported.
The Nature of Virus Infections

Why should we then worry about these data if it is such an innocuous virus? First, we should recall that probably the majority of all viral infections cause only slight or no symptoms in the infected individual. That viruses can cause disease is surely correct, but most viruses likely go unnoticed because only very few virologists take the pain to isolate viruses from healthy subjects or animals since it will be hard to motivate grant organizations to pay for such studies. The equation of virus = disease thus clearly suffers from a strong observation bias. However, there is a caveat to this reassuring statement about the benign nature of most viruses. Even if the virus is, through millions of years of coevolution, well adapted to its exploited animal and thus harmless to its natural host, it might be maladapted to an occasional heterologous host, where it can cause serious disease. And there is another disturbing point in this study. Simian foamy virus is a retrovirus like human T-lymphotropic viruses, which are associated with lymphoma and leukemia in humans. This infection is most prevalent in tropical forest regions of equatorial Africa, which suggests likewise a zoonotic origin.

AIDS as a Zoonosis?

Zoonosis is a human disease caused by the transmission of an animal virus. The simian origin of human T-lymphotropic viruses is now well accepted (Vandamme et al. 1998). To put the impact of zoonosis in the right perspective, there is also good evidence that AIDS is likewise a zoonosis (Hahn et al. 2000). The case is relatively clear for HIV-2: the human virus and that of monkeys (specifically that of sooty mangabeys) share a specific accessory protein and are phylogenetically closely related; sooty mangabeys are numerous in west African countries where HIV-2 infection is endemic. The animal host is infected with HIV-2-like virus at substantial frequency. Sooty mangabeys are frequently hunted for food, and orphans are kept as pets. Cumulatively, this evidence represents a smoking gun. A pandemic as in the case of HIV-1 did not occur because HIV-2 apparently lacks the capacity for efficient human-to-human spread outside of western Africa. The global human disease became a reality with the introduction of HIV-1 into the human population. In fact, it turned out to be much more difficult to trace the origin of HIV-1. Part of the problem is that HIV-1 is not a single virus but comprises three distinct virus groups termed M, N, and O. The predominant M group consists of more than 10 clades denoted as subtypes A to K. They represent at least three separate transmission events. Sequencing and phylogenetic analysis suggested a chimpanzee origin for HIV-1. This is not an unlikely origin since chimpanzee meat figures in African bushmeat markets. It is currently believed that the HIV-1 group M pandemic arose as a consequence of a single virus transmission event from a chimpanzee followed by a “starbust” radiation of numerous viral lineages in the new human host.
Origin and Spread of AIDS

An understanding of the origin of the AIDS pandemic is of substantial public health interest, but the epidemiological situation leading to the spread of the infection is complex and not yet clearly settled. Several events were tentatively associated with the origin of the AIDS epidemic. An interesting scenario is the association with commercial logging of tropical forests. This activity necessitates an intrusion into the bush and thus assures close contact with primates. The logging is accompanied by a network of other economical activities ranging from local commercial bushmeat trade over road construction to the arrival of sex workers. In combination, this setting could have paved the way for the spread of the original zoonotic infection. However, one should mention that alternative scenarios were proposed with respect to the origin of the AIDS pandemic. An especially dire hypothesis independent of the human quest for food and sex was the link with the polio vaccination campaign. According to this hypothesis, the polio vaccine might have been contaminated with the AIDS virus during the production of the vaccine on kidney cell cultures from chimpanzees held in tropical Africa. This scenario is, however, unlikely for two reasons. Old stocks of poliovirus vaccine used in Africa failed to provide evidence for HIV contamination and the timing of the diversification of the pandemic M group HIV-1 in the human population points to an introduction of the virus into the human population that predates the polio vaccination efforts in Africa. One elaborate computer study of full-length gene sequences of many M group isolates pointed to 1930 as the most likely date for this transmission event (Korber et al. 2000), a full 30 years before the start of polio vaccination in Africa. Furthermore, stored historical serum samples revealed a seropositive individual just before the onset of the polio vaccination campaign.

The Danger of Bushmeat

The bushmeat link is therefore a more logical hypothesis for the origin of AIDS. What makes bushmeat more dangerous than other meat used as a human food source? Humans have hunted for meat for ten thousands of years, and one should expect an adaptation of the human population to meat consumption and the associated problems of virus exposure. However, there are two peculiar problems with this form of bushmeat. The first problem is the fact that according to the archeological record, monkeys and apes did not figure in the human food list—any unusual wild animal as meat source represents a potential source for the transmission of potentially harmful viruses because humans had not adapted to live with the specific viruses associated with this particular meat source. Second, viruses are generally considered to be species specific and the likelihood of trans-species infections decreases with the phylogenetic distance separating the two animal host species. A primate virus is thus more likely to infect a human being than viruses infecting more distantly related mammals. This argument also explains why we are not victims to viral diseases transmitted from fish despite the fact that fishery is also a form of hunting of wild animals.
Infections of the Early Farmer

On the basis of these arguments, one would conclude that farming of domesticated animals is a safer source of animal meat for human consumption. At first glance this argument seems straightforward. However, its logic is not entirely watertight. On one side, animal husbandry is a relatively recent human occupation, and just 10,000 years ago, wild or half-wild animals were brought into close contact with humans. Physical proximity to an animal is a requisite for the transmission of an animal virus to us and vice versa. One can thus expect a period of extensive viral exchange between the pastoralist and its herd followed by a period of mutual adaptation to the respective heterologous viruses. Prominent biological anthropologists anticipate therefore that the early relationship between domesticated animals and humans during the Neolithic Revolution was not really a honeymoon. They postulate a lot of hardship from cross-species viral and bacterial infections during this phase. The greater stability of the food basis in the early farming societies when compared to the deteriorating hunting conditions during the Mesolithic was paid by the introduction of new viral diseases into the human population. We lack direct evidence for this process, but indirect data support this model.

Measles Virus

Take measles, a major killer of children on a global scale. Measles is a paradoxical viral infection. Humans are the only known host of measles, and historical records allowed tracing back measles for at least 2,000 years into human history. However, measles cannot be a genuine human infection. Classical epidemiological experiences on islands demonstrated that measles infection dies out on small islands; under this condition, measles propagation needs an external influx of humans (as occurred with the British troops during World War II on the Faeroer islands) to ignite a new wave of measles infection. To persist in the host species, measles needs to regularly encounter newly susceptible individuals who are contributed only by newborns since once infected with measles, it leaves a life-long immunity in humans. Only populations that exceed a quarter of a million individuals fulfill the requirement for measles transmission. Measles could thus only spread in the human population when agriculture provided in historical times the nutritional basis for supporting such a population size. Interestingly, the closest relative of measles is the rinderpest virus infecting bovids (Sharp 2002). Cattle are thus the likely origin of the human measles virus. It does not need a lot of fantasy to imagine the effect of newly introduced morbilliviruses into the human population during the Neolithic Revolution. Some anthropologists suspect that the rapid replacement of the Mesolithic hunter societies by the early farmer societies was caused by the collapse of the hunters when they met the farmers excreting measles. There is indirect evidence that this process of rapid replacement of one society by another occurred once again 500 years ago,
namely during the colonization of the New World by the Spaniards. As mentioned in a previous chapter, the literal melting of huge Indian armies in face of the small Spanish troops was probably caused or at least influenced by the effect of measles infections unknown to the autochthonous population. Notably, the American population did not domesticate cattle and thus lacked the experience with the rinderpest virus (reviewed in the lively book from J. Diamond “Guns, Germs and Steel” 1997).

Influenza Virus

One should not conclude from these two historical examples that animal farming is now a risk-free business for the human population with respect to viral infections. Dangers lurk in peculiar agricultural and market practices even today. The most threatening example is that of influenza viruses. Influenza A viruses possess a segmented single-stranded RNA genome, each encoding one of the eight viral proteins. Influenza A viruses infect humans, swine, horses, seals and a large variety of birds; the viruses are antigenically quite complex. Importantly, aquatic birds are the source of all influenza viruses in other species. Older studies on wild ducks in Canada demonstrated a number of remarkable observations: up to 20% of juvenile birds are infected with influenza virus when the birds congregate prior to migration, while none of the birds showed any symptoms of infection. This is commonly observed in influenza infection of wild birds. High viral loads are sent into the environment with the feces of the ducks leading to important but transient viral contamination of lakes.

Not all influenza infections are so benign. The catastrophic “Spanish Influenza” pandemic, which killed between 20 and 40 million people worldwide—more than all casualties in World War I—could be traced by sequence analysis to a virus from swine. This is a true detective story where virus hunters went through embedded historical tissue samples from soldiers who died in 1918 in military hospitals and to graves in the permafrost region. Since a swine epidemic occurred at the same time, the virus was probably transmitted from swine to human before efficient human-to-human transmission occurred. Notably, pigs serve as host for the replication of both avian and human influenza viruses. In a popular hypothesis, pigs are seen as a “mixing vessel” where genetic reassortants between avian and mammalian/human influenza viruses are created. Pigs may thus play a pivotal role in the generation and transmission of avian influenza viruses to humans.

Resurrection of the Flu

Exciting progress was made recently when the Spanish flu virus was literally raised out of its formalin (Taubenberger et al. 1997) and permafrost (Reid et al. 1999) grave. Initially this historical virus isolate led an in silico existence in the database. The analysis of its sequence corrected the older interpretation quoted in the preceding paragraph (Taubenberger et al. 2005). The polymerase sequences differed from the avian consensus sequence at only a small number of sites, making
it more likely that it was derived from an avian source shortly before the pandemic. Analysis of the now-completed genome of the 1918 influenza isolate suggested that this virus was not a reassortant virus like the later 1957 and 1968 strains, but entirely an avian-like virus that had adapted to humans. Notably, a number of changes observed in the 1918 isolate were also observed in the currently circulating, highly pathogenic H5N1 avian virus. The next step was even more breathtaking. Under high containment conditions, approved by NIH and CDC, researchers (under the protection of antiviral prophylaxis) used reverse genetics to unearth the 1918 virus not as a computer event, but as a real-life existence that could be propagated in cell culture as any other replication-competent influenza virus (Tumpey et al. 2005). The researchers wanted to grab the deadly secrets from the virus, and deadly it was. Mice started to lose weight and died a mere 3 days after infection. The damage was pronounced in the lungs, but the pathology remained restricted to the respiratory tract. The virus had apparently learned other dirty tricks: for example, it could be propagated in cell culture without the help of trypsin. Ordinary influenza A viruses need trypsin for the proteolytic cleavage of their hemagglutinin. In mice, virus titers of the 1918 isolate skyrocketed in the lung; likewise the lethal doses dropped by at least a factor of hundred in comparison with reference influenza viruses. In addition, the 1918 virus was lethal for fertile chicken eggs, a pathogenic feature of avian H1N1 viruses.

Why China?

Epidemiologists have always wondered why China is the source of new pandemic influenza strains. When new pandemic strains replaced the predominant H1N1 subtype (H refers to the hemagglutinin and N to a neuraminidase, the two major external proteins of the virus, and the numbers to different serotypes), like in the 1957 Asian flu with an H2N2 subtype or the 1977 Hong Kong H3N2 subtype, avian genes contributed to the new pandemic strains. Virologists suspected that the access of pigs to ponds with ducks as practised in traditional family animal rearing in China might explain the geographical predilection for the emergence of new influenza virus subtypes in this region.

The Avian Flu

The “Hannibal ante portas” is still a clear warning in our days: avian influenza strains are clearly knocking at our doors. This became evident in 1997 in Hong Kong when 18 confirmed cases and six deaths were reported that could be traced to the avian H5N1 subtypes (de Jong et al. 1997). The H5N1 virus was nonpathogenic in duck, but highly pathogenic in chicken and humans. It was prevalent in chicken from live bird markets in Hong Kong, but failed to transmit efficiently from human to human. A potential catastrophe was prevented by a massive culling of 1.5 million chicken within 3 days and the cleaning of markets and the separation of markets for live chicken and live aquatic birds. Later the authorities banned ducks, geese, and quails from the markets and imposed
vaccination with an inactivated H5N1 vaccine for all domestic and imported poultry. These measures were successful and no H5N1 viruses were isolated from domestic poultry or humans in Hong Kong since 2004 (Webster and Hulse 2005). However, they have not stamped out H5N1 influenza—since December 2004, 41 people have been infected with H5N1 in Vietnam, where both commercial poultry farming and backyard poultry are expanding. The strains are highly virulent for humans and caused at least 16 deaths (Tran et al. 2004). H5N1 is now endemic in ducks and highly virulent forms were now reported in wild waterfowl such as geese at the Qinghai Lake, where large number of dead geese were counted (Chen et al. 2005).

Geese showed not only diarrhea, but also neurological signs such as tremor and opisthotonus (a retroflection of the head). Brain lesions and pancreatic necrosis were seen by the veterinary pathologists. Virus was isolated from the viscera, the brain, and also from the oropharynx and the cloaca of sick and dead animals. The latter two routes of virus excretion are directly relevant for the transmission of the virus. Infected gulls (Larus) were also detected at this lake, which is significant since gulls eat carrion and might thus get infected from dead geese (Liu et al. 2005; Figure 7.2).

The importance of this observation, which was disputed by the Chinese government (Butler 2005), is given by the fact that this lake in western China is an important aggregation and breeding ground before the animals migrate southward to Myanmar or over the Himalayas to India. Some Asian geese take even a migratory route over Europe. Because infected birds excrete influenza viruses with their feces, the droppings of migratory birds have a great potential to spread the disease. As cats and tigers turned out to be susceptible to these avian viruses, the potential of transspecies infection is clearly given. The situation is compounded by the dynamic nature of the H5N1 genotypes in eastern Asia. Since its isolation in 1999 from a goose in Guandong, China, numerous reassortants have been observed over the last 5 years; many muster genes from up to four different viral sources via “antigenic shift.” Also the individual genes showed a high rate of amino acid substitutions (“antigenic drift”); the virus is thus clearly not in a stasis, but actively evolving. In contrast to the “starburst” feature of the phylogenetic tree from HIV-1 M subtypes, the H5N1 gene sequences show a successive replacement of older by newer types as if the virus goes repetitively through genetic bottlenecks in its adaptation to new hosts and ecological settings (Guan et al. 2004).

Many industrialized countries are now preparing for the potentiality of an H5N1 epidemic. Prominent veterinary virologists such as Albert Osterhaus from Rotterdam University are ringing the alarm bell that migratory birds might spread influenza virus on their path between the northern summer and the southern winter quarters. Casualties with H5N1 in wild waterfowl are now also seen in Europe, where swans seem to be especially afflicted. Migratory birds in the winter residence of Nigeria showed individuals infected with H5N1. Likewise, eastern Turkey experienced an outbreak of H5N1 flu in poultry, and persons in close contact with infected chicken (Figure 7.3) contracted the disease
Figure 7.2. Herring gull (*Larus argentatus*), family Laridae, order Charadriiformes, in the foreground.

and some died of it. The WHO has listed 152 laboratory-confirmed cases of human H5N1 infection over the last 2 years with a fatality rate of 50%. So far, most cases still involve direct transmission from poultry to humans. However, the first probable cases of human-to-human transmission have in the meanwhile
Figure 7.3. Chicken (Gallus gallus) represent today a major source of animal proteins to human nutrition by providing meat and eggs, both in industrialized and developing countries. The figure shows the likely ancestor of the domesticated chicken, G. gallus ferruginous, from Bankiva, Malaysia. Chicken were initially not raised for meat or egg production, but held for cockfighting. The food use of chicken was propagated by chicken raisers only at about 1900 and became important after 1920. 

been reported (Ungchusak et al. 2005). If this mode of transmission will become established, the specter of a pandemic flu outbreak in the human population becomes possible. Are we prepared?

On Models and Superspreaders

Epidemiologists made computer calculations to simulate the impact of different control measures on an epidemic taking Thailand as a model case (Ferguson et al. 2005). The critical parameter is the basic reproduction number $R_0$, a transmissibility parameter. It is defined as the average number of secondary cases generated by a typical primary case in an entirely susceptible population. If $R_0 > 1$, an infection will spread, with higher values it will explode, whereas with $R_0 < 1$, the chain of transmission will die out. To get $R_0$ below 1, public health has three principle measures. First, one can reduce the contact rate in the population, e.g., by closing schools or airports. Second, one can reduce the infectiousness of the infected person, e.g., through drug treatment or classically by quarantine. Third, one can reduce the susceptibility of uninfected individuals by antiviral prophylaxis or vaccination. You might wonder why I detail the infection process here, but do not forget we have here an especially insidious predator (the virus)—prey (us) pair, where the predator multiplies after each successful predation event. The simulation showed that with $R_0 = 1.5$, the infection remained for 30
days around the seeding location of human-to-human transmission, swept over the country in 90 days, became thereafter international, and was over in the model country at day 200, when 33% of the population became infected. With $R_0 = 1.8$, the infection rate is 50%. The authors for the Thailand simulation found that a combination of geographically targeted prophylaxis and social distance measures could wipe out the epidemic if cases are rapidly identified and 3 million courses of antiviral drugs are stockpiled. The drug now plays the role of an antinutrient or a chemical armor against predation. How tight is this strategy? In Europe several governments have started to stockpile drugs such as oseltamivir for the prophylaxis of health personnel and for the treatment of patients. This drug is a neuraminidase inhibitor and impedes the spreading of the virus through the body of the infected person. With antimicrobial drugs, the development of drug resistance is a problem. In that respect, a case report from Vietnam is alerting (Le et al. 2005). A 14-year-old girl without contact with poultry cared for her 21-year-old brother, who suffered from an H5N1 infection. The girl received oseltamivir prophylactically, but nevertheless contracted the virus apparently from her brother (some viral clones were sequence identical). She was then treated with oseltamivir and recovered. However, virologists isolated a drug-resistant H5N1 virus from her that showed a characteristic neuraminidase mutation that is known to confer resistance to this drug. The simulation depended on the value anticipated for $R_0$. However, the model also varies with different distributions of the $R_0$ value in an infected population. For sexually transmitted and vector-borne disease, epidemiologists used the “20/80% rule,” according to which 20% of the cases cause 80% of the transmissions. When evaluating the transmission data of the relatively well-investigated SARS epidemic, scientists identified individuals with exceptionally high $R_0$ values, who were called superspreaders. This skew in the $R_0$ distribution led to entirely new transmission dynamics for epidemics (Lloyd-Smith et al. 2005).

Avian Flu Vaccines

Finally, a word on vaccines. Currently H5N1 vaccine production depends on a supply of embryonated eggs to produce inactivated subvirion vaccines (Webby et al. 2004). The virus produced on two eggs is needed to immunize one person; this is much cheaper than producing flu vaccine on cell culture. However, in the case of a pandemic with avian flu, chicken might be the critical transmitter and the authorities will call for massive destruction of chicken flocks. In view of the hundred millions of doses needed, there might not be enough laying hens around to satisfy the demand for embryonated eggs. Furthermore, H5N1 is quickly lethal for the embryonic egg—it might kill the egg without producing a high harvest of progeny virus. Ironically, rescue might come from another virus. Scientists from the CDC in Atlanta introduced the H5 hemagglutinin into a replication-deficient human adenovirus vector. It produced both humoral and cell-mediated immune responses against various H5N1 strains in mice and protected the animals against lethal challenge with flu virus (Hoelscher et al. 2006).
We are not Alone

The take-home message is clear. Despite decades of active research into influenza viruses, we are still at the mercy of this dynamic virus that seems to look for breaches in the species barrier. A recent example is the transmission of H3N8 equine influenza virus to dogs. Veterinarians became aware of this problem after virological investigations in an outbreak of severe respiratory disease with high mortality affecting greyhounds from various US racing grounds. The virus isolated from the affected dogs was according to genome sequence analysis a clear-cut equine virus. The viral hemagglutinin showed adaptation to the new host by changing a few critical amino acid positions. Even more disturbing was the observation that according to serological evidence, the equine virus transgressor had sneaked into the pet dog population (Crawford et al. 2005). Apparently, the viral empire strikes vigorously back, and we are getting cornered by influenza viruses coming from different fronts.

Natural is Not Necessarily Healthy

We are well advised to keep an open eye on these avian influenza viruses since they could become a threat to human health comparable to the AIDS pandemic with millions of deaths. With safer sex measures and blood screening, the AIDS pandemic can at least theoretically be contained in the industrialized world; a pandemic influenza will be much more contagious and thus more disruptive for the world economy as already demonstrated by the smaller SARS epidemic. *It is important that a wider public realizes that these widely known viral infections are intimately linked to backyard animal rearing combined with the dangers of live animal markets.* In Europe there are at present strong currents with the consumer to appreciate food from natural sources in the neighborhood that are minimally processed over products from established agricultural practices that are processed by the food industry. Many biologists think that these consumers commit an important error when equating “natural” products with healthy products. They are probably influenced by romantic feelings that were so prevalent in different epochs of European art and thinking. During those periods, ideals of the antiquity and Mother Nature were painted that never existed except in the heads of artists and philosophers. Also the Christian faith with its belief in a caring father and the heavenly commandment to subject nature to our order might have influenced this benign perception of nature. Even if I used in several passages of the book the metaphor of “Mother Nature,” we should not anticipate a caring principle behind this name. As stressed in another context, the “Mother Nature” picture corresponds more to the Hindu trinity of gods combining at the same time the creation of the new (Brahma), the maintenance of the existing (Vishnu), and also the destruction of the old (Shiva). Romantic westerners forget all too easily that Shiva is part of our “Mother Nature” concept. Prominent veterinary virologists such as Albert Osterhaus suspect that free-range hens on commercial European poultry farms could play a crucial role in the transmission of avian H5N1 influenza infection from migratory birds to the human population.
One gets the impression that these production facilities are maintained for a romantic feeling about a species-adequate animal husbandry or a sense of guilt toward fellow creatures, which we maintain as a food source. Europeans are there in a dilemma since the Christian belief did not formulate special commands toward animals. Ecology-oriented biologists claim more rights for wild animal life frequently for a purely egoistic reason, namely a concern for the future of the human civilization, but are less expressive with domesticated animal rights. The public knows subconsciously that a Rousseau-type “retour à la nature” will not lead domesticated animals and agriculture back into a terrestrial paradise. The Old Testament vision of the prophet where the lion lies with the sheep sets the stage for eschatological hopes for a world freed from the quest for food, but at the same time, the public knows that such a world cannot be populated with animals selected by evolution on the planet earth.

**Problems of Food Safety: BSE**

The Underused Cattle

We mentioned prion disease as a danger of cannibalism in remote New Guinea. Prion disease has now shaken the confidence in public health systems in Europe. It is difficult to refrain from political undertones and to restrict the discussion to basic scientific arguments when recounting the BSE crisis in Europe. I will try a neutral account. In the opening chapter, I mentioned that the European culture and all overseas cultures built on its ideals are deeply rooted on a cattle cult. Dairy and beef industries are important parts of the economic life and make a major part of the dietary identity of its people. However, only few parts of cattle actually go into the human food chain. Human imagination found other uses: less appreciated organs go into pet food, hides into the leather industry, bones into gelatin production for the film industry and as one of the most important binders into the food industry. This current practice has not created any major problems. Yet, still substantial parts of the body of the cattle remain unused, and technologists searched for alternative use of waste material like horns and hoofs and offal. If all this is crushed to its elemental biochemical constituents, the resulting “bone meal” should be a valuable addition to the feed of agronomically important animals. Apparently, no biologists trained in evolutionary and ecological thinking participated in the development of these rendering industries and the technologists overlooked that cattle not only were turned into carnivores when this type of material entered their feed, but actually became cannibals. As strict herbivores, cattle’s only carnivore activity is when the dam eats its own placenta. Unfortunately, the lessons of the ritual cannibalism in the Fore tribe were not taken as a warning. Veterinary epidemiologists now paint the same scheme as in kuru. Perhaps one rare individual developed a spontaneous form of this disease, which would have ended with the death of that animal. By the feeding of bone meal, the agent was introduced into the food chain. In cattle the infected animal had a chance to enter into the rendering system and the infectivity could be amplified.
A Short History of BSE

Epidemiologists have postulated that other factors added to this scenario to explain why the BSE epidemic occurred only in the 1990s. One interesting, but not proven argument is the energy crisis which forced the low-profit rendering industry to change their technology to decrease the price of the rendering process. This change could have allowed the infectious agent to slip into the product. Whatever the exact epidemiological explanation, the fact is that the number of infected animals skyrocketed in Great Britain. The distribution of cases—or its reporting practice—was patchy in continental Europe. Then the British government reacted with a ban on the feeding of bone meal, made BSE a notifiable disease, explored the distribution of the infectious agent in the body of clinically affected animals, and declared several organs like the brain unfit for human consumption. The best scientific minds of British epidemiology and biomathematicians as well as veterinary and human neuropathologists addressed the problem and came up with discoveries that impressed the scientific observers in the food industry, which had never before been confronted with such an enigmatic agent in their food safety evaluation systems. Overall, the research in BSE is a scientific success story and—although with some delay—the BSE cases came dramatically down, verifying the correctness of the scientific assumptions.

Communication Problems

However, from the communication side the BSE crisis was a disaster that destroyed the confidence in the public health systems. Personally, I think that the grandmother argument was neglected. Scientists and politicians alike underestimated the deep emotional fears of humans toward food and the risks of food poisoning from the hunter-gatherer phase of human evolution, which represents the overwhelming part of human evolution. Only recently have humans started with agriculture where the risk of poisonous plants has been dramatically reduced. However, the safest food can still be spoiled by microbial overgrowth, therefore the threat of food poisoning remained real over most of the historical time and experienced a dramatic decrease only very recently with the industrialization of food processing. The lay public literally used their “gut feeling” and not their brain feeling to address this safety issue. Furthermore, they got in the wake of the BSE crisis another crisis, which confirmed the bleak presage that BSE is potentially only the top of an iceberg.

How Safe is Meat?

I will illustrate the problem of food safety with three recent publications asking for the presence of prions in skeletal muscle of infected animals. German scientists used transgenic mice expressing bovine prion protein (Buschmann and Groschup 2005). These mice turned out to be good sentinels for prion detection as they were 10 times more sensitive toward bovine prion infectivity than cattle. When they searched different anatomical sites in BSE-afflicted cows for infectivity, they found it only in the central and peripheral nervous system and not in
lymphatic tissue. The only exception was the Peyer’s patches of the distal ileum, which is most likely the site of entry for BSE infectivity. Apparently, upon oral exposure to the infectious agent the BSE infectivity spreads centripetally to the central nervous system via the enteric nervous system and peripheral nerves. Amniotic fluid and colostrum lacked prion infectivity suggesting that neither intrauterine transmission nor milk feeding is a mode for vertical transmission of the disease from the dam to the calf. With a single exception, which might represent an experimental error, no muscle samples from BSE cows transmitted the disease to transgenic mice. However, the presence of prions in muscle meat cannot be dismissed. In fact, in North American mule deer, kept in captivity for meat production, a prion disease was described. Animals afflicted with this chronic wasting disease demonstrated regularly prion infectivity in muscle tissue that could be transmitted to mice. The longer and more variable incubation period in the inoculated mice compared to those exposed to brain samples suggested lower infectivity titers in the muscle than in the brain (Angers et al. 2006). While these data could suggest some caution toward mule deer meat consumption, scientists knew that sheep afflicted with a prion disease called scrapie show a widespread anatomical distribution of infectivity. In naturally infected sheep, the PrP\textsuperscript{Sc} was detected in muscles several months before clinical disease onset (Andreoletti et al. 2004). The titer was 5,000-fold lower than in brain, and the muscle infectivity was concentrated over spindles, highly innervated structures that ensure muscle proprioception. As scrapie is a sheep disease known in Britain for more than two centuries, sheep-derived PrP\textsuperscript{Sc} has in appreciable quantities entered the human food chain. Nevertheless, on the basis of epidemiological data dietary exposure to scrapie-infected sheep is currently considered nonhazardous to humans. However, one should not trust this reassuring evidence from sheep too much as there is good epidemiological evidence that BSE has entered the human population as discussed next.

The New Threat

The new disease is called in scientific slang the vCJD epidemic. The abbreviation stands for variant Creutzfeldt–Jacob disease, which most likely represents the intrusion of the BSE agent into the human population. Again, on the scientific side it represents a success story. Only few countries outside of Britain would have noticed this new disease—only scrupulous screening of death certificates and enormous neuropathological dedication led to the definition of this disease in young adults. Numerically, the toll of this disease is not yet heavy and happily the rate of new case identification is decreasing. However, the jury is still out because the long incubation period of the transmissible spongiform encephalopathies does not yet allow the conclusion that we have already seen most of the food-mediated transfer of BSE into the human population. Actually, the greater medical concern nowadays is that BSE crept subclinically into the human population (millions of people were exposed to BSE-tainted food products, but only a few dozen developed vCJD until now) and can now be further transmitted by medical “cannibalism” (blood transfusion, organ transplantation). There is precedence to
iatrogenic transmission of CJD when stunted children were treated with growth hormone that was not produced by genetic engineering, but was isolated from the pituitary gland of a large number of human cadavers. Unfortunately, one dead donor was incubating CJD and infected a sizable number of French children.

An Unusual Pathogen: The Prion Hypothesis

Kuru is the last, apocalyptic chapter of virology textbooks like “Field’s Virology,” but it fits this classification as a virus only with the original Latin meaning of virus as a poison, not the modern definition of a viral particle. Despite substantial effort and heated discussions at scientific conferences that bordered on political or religious strife, no genetic material in the form of nucleic acids could be associated with the infectious agent. This lack of a genome could also well explain the disturbing resistance of the agent toward chemical and physical actions. Standard medical sterilization procedures were insufficient to destroy the infectivity. To come to grip with a model for this mysterious disease, S. Prusiner developed over the years the prion hypothesis, which is nothing less than a new class of infectious agent consisting of a rogue protein with an aberrant 3-D conformation called PrP\textsuperscript{Sc}. PrP\textsuperscript{Sc} imparts its misshapen conformation to its normal cellular counterpart, the PrP\textsuperscript{C} protein. PrP\textsuperscript{C} is thus misfolded under the influence of PrP\textsuperscript{Sc} and can then transfer the newly acquired pathological conformation to further normal PrP\textsuperscript{C} proteins. In this way PrP\textsuperscript{Sc} can “replicate” and become an infectious agent.

Opponents maintained, for example, a virino hypothesis, i.e., an agent containing its own nucleic acid enveloped in host-encoded proteins. In a recent editorial, prion disease researchers compared this discussion with a key paper from 1840 by Joseph Henle (Zou and Ganbetti 2005). Henle proposed at this early time that infectious diseases are caused by “contagia animate”; we would today say microbes, and not by miasma, poisoned air. Actually, the name malaria still in use today reflects this old and outdated “bad air” hypothesis. Now biologists are striving to fulfill Koch’s postulates for prions. Some progress along this line was achieved recently when a truncated fragment of mouse PrP\textsuperscript{C} protein could be misfolded in vitro into β sheet-rich fibrils. After intracerebral inoculation, these fibrils could cause disease, albeit only after a long incubation period and in transgenic mice highly overexpressing the truncated PrP\textsuperscript{C} protein (Legname et al. 2004). Now, scientists mixed an excess of PrP\textsuperscript{C} with small amounts of PrP\textsuperscript{Sc} and conversion of PrP\textsuperscript{C} was observed. The trick was that they now used sonication that disrupted the new fibrils and they added the result of the first round of amplification into a new test tube containing fresh PrP\textsuperscript{C}. Like in a type of PCR, this cycle of incubation, PrP\textsuperscript{C} to PrP\textsuperscript{Sc} conversion, sonication, and transfer to a fresh tube was repeated many times. The researchers calculated that the initial brain inoculum contained perhaps $10^{11}$ molecules of PrP\textsuperscript{Sc}, but the series of amplifications implicated a dilution of $10^{-40}$. It is thus physically impossible that the initial material is transferred into the last tube. Nevertheless, the last tube led to a scrapie disease in wild-type hamsters that was identical in all respects to that produced by the initial infectious brain material (Castilla et al. 2005).
Critics might still maintain that some type of RNA might have been amplified along with PrP\(\text{Sc}\), but this hypothesis lacks any experimental evidence and also appears somewhat farfetched.

Going for our Blood

Real-life Draculas

On Vampires: Real and Imagined

As a scientist you might wonder about the boom of horror and disaster films, which fill the box offices today. Some people want to see it as a sign of degeneration of our civilization, which begs its end by anticipating its annihilation. However, as a scientist you should differentiate your judgment. Some films (e.g., Armageddon, Outbreak) have such a clear scientific core message that you can easily quote articles from scientific journals that deal with the same subject. Other films seem to appeal only to our lower instincts. Yet for a biologist, appealing to instincts is not necessarily negative. Some films call on what one could characterize as a collective memory of humankind. Take as example perhaps the oldest and most successful subject of horror films. The plot is in Transylvania—already the naming of this real province in Romania gives strange feelings, a mixture of fright and delight typical for these types of films. And as in fairy tales there is a real-life nucleus to the story. Briefly, a wicked Romanian count transforms every night into a bat-like blood-sucking creature, which rests in its grave during daytime. It can only be put to eternal rest when a wooden peg is pierced through its heart. Prominent in the fight against the evil is a scientist, actually a zoologist specialized in bats and who uses garlic as a vampire repellent. Actually you can identify historical and real-life aspects in this story. A zoologist will think of blood-sucking bats of the family Phyllostomidae (also called “vampires” by zoologists). Already Alexander von Humboldt reported on blood-sucking bats during his travels through South America, following accounts from physicians accompanying Spanish soldiers in the New World. Horses were the main victims: bats sucked blood from superficial vessels in the skin, which could still bleed the next morning. The bats punched out a small area of the skin—the wounds were only harmful when flies subsequently laid their eggs into the wound. Humans suffered mainly from painless and generally harmless bites on the toes and the nose. Blood-sucking by bats is only prominent in Latin America and so rare in Europe that it is an unlikely source for the Dracula legend. In fact, Romanians still remember a cruel king with a Latinized name of Dracula. He impaled Turkish prisoners of war, hence perhaps the origin of the wooden dagger through the heart. During the period of the reign of Habsburg Empress Maria-Theresia, rumors of vampires reached Vienna and the Empress sent her court physician to Romania—the core for the legend of the professor fighting against the vampires. Actually, he reported that the suspected cadavers were vividly colored with a reddish skin and when the peg was pierced into the
body by frightened fellow villagers, an intensively red liquid oozed out giving the impression of liquid blood. Modern-day pathologists suspect that these corpses suffered from a bacterial putrefaction process where the red color came from a bacterial pigment. The piercing with the wooden dagger would have caused a splash of the bacterial culture, which could have infected the bystanders of this rite, who would have suffered similar putrefaction after death from a bacterial infection.

Mosquito Biting

Tragically, Dracula is still amongst us and when the night falls he sets out to get his blood meal and to spread death in the world. However, it is neither a devil nor a bat, but a little fly or in Spanish a “mosquito” (Figure 7.4). Their mouth parts seem to be tailor-made for blood sucking. The proboscis consists of two stylets that slide against one another when piercing the skin in search for a blood vessel. When not directly successful, the mosquito can retreat the needle and change the angle for injection to have a second try in its quest for blood. Inside the proboscis are two hollow tubes. Through one of them the animal injects its saliva, through the other it draws the blood of the victim. This double-barrel high-tech surgical device uses a lot of pharmacology during its action. To prevent blood clotting, which would clog the feeding tube, the insect injects with its saliva an anticoagulant. The mosquito also injects antiinflammatory chemicals. You might hear the insect or see it when it is sitting on your skin, but you will not feel it during the blood transfusion. Even this elegant feeding device needs more than a minute for a blood meal. During that process you will not experience a pain reaction, which is important for the animal to escape unharmed. Once away, an intense irritation will set in, which leaves you an itching memory. At that moment the culprit is already away. However, it has not gone far—it might sit on the wall or the ceiling of your room. This should not surprise us: the mosquito has taken a substantial amount of blood, which increased its weight by a factor of four (Budiansky 2002). Small wonder that rest and digestion of the protein-rich blood meal and absorption of the nutrients are now the priority.

Blood Meal

The mosquito surrounds the stolen blood by a peritrophic matrix consisting of protein and chitin. Proteases secreted by the insect gut then penetrate this matrix and liberate smaller hydrolysis products from the blood. Microvilli-bound enzymes finish the digestion before absorption by the midgut cells. The nutrients travel to the fat body, roughly corresponding to a mixture of the liver and adipose tissue of our body, where egg proteins and lipids are synthesized. These synthesis products then go via the hemolymph to the insect ovary, where they are used for egg development, which takes 3 days until oviposition. During this time, the female mosquito takes no food (Holt et al. 2002). After egg
laying, a new victim is searched for a new blood meal since only the energy-rich blood can sustain this demanding reproductive activity. This becomes clear when looking at *Culex pipiens* populations (the mosquito known to Europeans and North Americans) sporting distinct feeding habits. Subpopulations that do not feed on blood lay only about 60 eggs once in their lifetime. Blood-seeking subpopulations in contrast can produce 400 eggs per blood meal and this happens repeatedly during their life (Budiansky 2002). Only egg-laying females suck blood. Early after metamorphosis into an adult mosquito, the animals take sugar meals from plant nectar to maintain basal metabolism and to power the flight. Flying is important to find a mate and the victim for the first blood sucking. As producing sperms is a low-energy business, male mosquitoes remain lifelong vegetarians.

Chemical Cues

Mosquitoes come in many forms and are not limited to specific climate zones. In fact during the short summer period, Artic zones become so densely populated by mosquitoes that dead caribous lacking blood were described. Some mosquitoes hunt during daytime and use visual cues; some even chase their victims. However, most mosquitoes hunt in the night and have thus to rely on other senses to find their prey. Mosquitoes differ in their searching profile, which determines their host specificity. Commonly, they are attracted by a 37°C skin temperature,
moisture, CO₂, and specific odors. The odor preference was tested using olfactometers. The notorious *Anopheles gambiae* mosquito bites its human victims on the feet and ankles. It is a well-known observation that the human foot odor bears a remarkable resemblance to a few strong-smelling cheese specialties. This parallel is not fortuitous: *Brevibacteria* are found in the moist clefts between the toes as well as in strong-smelling cheese types. Currently, devices that emit such odors to deter mosquitoes from their human victims are in development (Enserink 2002).

The *Anopheles* genome

The design of antimosquito measures is now helped by the sequence of the *A. gambiae* genome (Holt et al. 2002). The size of the genome of this insect is 278 Mb and is substantially larger than that of the fruit fly *Drosophila melanogaster*, which shows only a 122-Mb genome size. However, both genomes show a comparable number of about 13,000 genes. The difference in size is thus largely due to variation in intergenic DNA. Half of the genes are orthologs and share an average sequence identity of 56% (Zdobnov et al. 2002). This percentage corresponds to the genetic distance separating humans from the pufferfish. This is a surprising observation since the two dipterans (the zoological term for these two-winged insects) diverged only 250 My ago, while the two vertebrates separated from a common ancestor 450 My ago. These two insects apparently diverged on the fast lane. Inspection of the *Anopheles* genome identified 276 G protein-coupled receptor genes that play roles in many pathways affecting nearly all aspects of the mosquito’s life cycle (Hill et al. 2002). Especially prominent are chemosensory receptors: nearly 160 genes were classified as either odorant or gustatory receptor genes. Thus about 1.5% of the proteome is dedicated to these chemical senses. Impressive as this percentage appears, it is not unusual. Animals have to deploy substantial care for finding food and deciding on its suitability for eating. Mosquitoes are thus not an exception in nature; *D. melanogaster*, which lives on rotting fruits, shows a comparable percentage of chemosensory genes. The nematode *Caenorhabditis elegans* uses even 6% of its proteome for chemical sensing. However, the DNA blueprint is essentially static information, which allows only limited inferences on the involvement of the different genes in the various life processes. This DNA sequence information can now be made dynamic by mRNA expression analysis. When this is done for blood-fed and nonblood-fed *Anopheles*, researchers found about 100 upregulated and 70 downregulated genes. Prominent under the upregulated genes were protein digestion and protein and lipid synthesis genes. As expected, muscle-related genes and genes encoding enzymes for sugar meal digestion were downregulated (Holt et al. 2002).

The Mosquito as a Vector

Bioinformatic analysis also identified 242 genes in the *Anopheles* genome involved in innate immunity. Adaptive immunity does not occur in insects and
is in fact limited to chordates. This figure compares to 185 corresponding genes in *Drosophila*. The excess of the mosquito genes is especially marked in the recognition category of innate immunity genes (Christophides et al. 2002). This expansion of immunity genes probably reflects the different lifestyles of both dipters. Here we touch an important point of the mosquito’s feeding strategy, which has crucial medical implications. When feeding, mosquitoes become exposed to human pathogens, and some of them actually use the mosquito as a vector. If the mosquito wants to survive on its nutritious, but potentially dangerous blood meal, it must find an answer against getting infected by the many blood-borne pathogens. The pathogen has of course no interest to kill the host, it only searches for a lift from one to the next human victim as its transmission relies on cycling between the vertebrate and the insect host. There are many mosquito-borne diseases, but the numerically most prominent and the most hideous is malaria. In fact, *A. gambiae* carries the deadliest of the malarial parasites, namely *Plasmodium falciparum*.

**Hitchhiking the Blood Sucker**

*Plasmodium*: Life Cycle

Malaria is endemic in more than 100 countries girdling the equatorial zone of the world. It accounts for 300 million cases and 1 million deaths each year, with most deaths occurring in African children. Unfortunately, this is not the only mosquito-borne disease. Mosquitoes transmit dengue, yellow fever, and Japanese encephalitis virus and even parasitic worms causing lymphatic filariasis. The latest addition to this list is the West Nile virus in the United States—industrial countries are thus not spared. The *Plasmodium* parasite (Figure 7.5) leads a complicated life that puzzled scientists for over 100 years since its role in malaria was established. Already the naming of the different stages is complicated (Wirth 2002). When injected by the mosquito the *Plasmodium* is in the sporozoite stage, which travels to the liver. There it goes through several stages to become merozoites that infect the red blood cell. In this cell the *Plasmodium* develops into a so-called trophozoite. As the name indicates this is the major feeding stage of the *Plasmodium*. Actually all clinical signs of malaria such as fever, chills, anemia, and cerebral malaria can be explained by the altered state of the infected red blood cell. The trophozoite then goes again through a merozoite stage. *Plasmodium* is released from disrupted red blood cells only to infect new red blood cells. Finally, up to 10% of the erythrocytes of the patient is infected with the malarial parasite. If the *Plasmodium* does not develop further, it would be locked in the infected patient. Therefore, the merozoites in some red blood cells still take another turn: they develop into gametocytes. With the next blood meal mosquitoes ingest these cells. Within the gut of the insect the gametocytes develop into male and female gametes (reproductive cells), which fuse to a zygote. This becomes an ookinete, the strange name means “moving egg” and describes the traveling of this cell through the gut wall. It transforms then into an
Figure 7.5. The life cycle of *Plasmodium vivax* starts in humans with the injection of the sporozoite (*I*) from the saliva of the mosquito. The sporozoites enter liver cells, where they mature into tissue schizonts (2–5), which rupture to release merozoites (6). Once within the bloodstream, merozoites invade red blood cells and either mature to erythrocyte parasites (ring trophozoite → schizont → merozoites, not depicted) or they differentiate into sexual forms: male gametocytes (*7b–11b*) and female gametocytes (*7a–11a*). However, the protists proceed with the following sexual stages only in the next mosquito vector. Within the mosquito midgut, the haploid male gametocyte loses its flagellum to become a male gamete (*11b*), and fertilizes a haploid female gamete (*9a*) to produce a diploid zygote (*12*). The diploid zygote then transforms into an ookinete (*13*), which invades the gut of the mosquito to become an oocyst (*14*). It then undergoes a meiotic reduction division to produce the haploid sporozoites (*15–18*) that migrate to the salivary gland (*19*) to complete the infection cycle. The sporozoite then infects the next human victim.
oocyst, which finally bursts releasing many sporozoites that travel to the salivary gland of the biting insect.

Fungi as a Weapon

The cycle in the insect takes about two weeks to complete. This is a relatively long period and thus a potential Achilles’ heel of the parasite. If something happens to the mosquito vector during this period, the parasite is stopped. Therefore, even slow-killing agents can have a great effect on malarial transmission. One of the most recent antimalaria approaches targets just this window of opportunity. Researchers sprayed two entomopathogenic fungi on surfaces commonly used by mosquitoes in the postfeeding rest period. Mortality was observed only after a week, but reached 90% before the ingested gametocytes could develop into newly infectious sporozoites. The sporulating fungi were quicker and had grown out of the mosquito cadavers in 70% of the infected animals. In addition the appetite of the infected mosquitoes for blood was lost even before that (Blanford et al. 2005). Importantly, these fungi can infect and kill the mosquito without being ingested. Contact with the mosquito just for the 1-day rest period of the mosquito after a blood meal was sufficient to infect the vector. Even more importantly, infections of the mosquitoes with the fungi were observed under real-life conditions during a field study in Tanzania (Scholte et al. 2005).

Herbal Medicines?

Alternatives in the fight against the insect vector are chemical repellents either applied to bed nets or directly on the skin. Some chemicals showed a dose-dependent protection from insect bites with standardized mosquitoes. Many consumers in industrialized countries are now weary about chemicals and prefer plant extracts believing that this is natural (although this is ultimately of course also chemical). And indeed soybean oil had a moderately good repellent effect, rehabilitating the good old folklore of using garlic against count Dracula. However, not all plants will do the job: Citronella extracts, widely used against mosquito bites in industrialized countries, turned out to be inefficient in controlled tests (Fradin and Day 2002). The ecology-minded consumer should therefore think twice about soft medicine approaches against vector-borne diseases. Clinical tests with herbal extracts have more negative outcomes than just Citronella plants. Echinacea plants recently joined this list when their extract turned out to be of no effect against rhinovirus infection (Turner et al. 2005).

Plasmodium Genomics

Now biologists have a great aid in their fight against malaria: the 23-Mb-large genome of *P. falciparum* has been determined (Gardner et al. 2002). As genomics is a much more powerful tool when it can be done on a comparative basis, the sequence of the rodent malarial parasite *Plasmodium yoelii* (Carlton et al.
2002) adds substantially to the antimalaria approaches, not least because it puts the analysis of the mouse malaria model on a firmer footing. *P. falciparum* musters 5,300 genes, slightly less than the baker’s yeast, which shows 5,800 genes. If you look at the many lives of the parasite, it is a quite remarkable feat to achieve this with so few genes. However, you should not forget: yeasts are not so primitive, and it needs only a little more than twice as many genes to make a mosquito. Surprisingly, humans are made with less than 5–10 times the amount of genes you find in a baker’s yeast, although at the level of gene products the factor is 15. It seems that nature constantly humiliates human pride. After Copernicus’ and Darwin’s revelations, we are taught modesty again by geneticists. However, there are also positive aspects to these humbling number games. The genomes of man, mosquito, and malarial parasite are now available; the tools are there to attack the killer by systematic approaches. Most of these genomics and postgenomics approaches are concerned with targets for drugs or vaccine development. As this book is written under an “eating” heading, I will illustrate the power of these approaches for the metabolism of the parasite.

**Plasmodium Metabolism**

About 700 enzymes were annotated in the *P. falciparum* genome, about 14% of all predicted proteins. This is a small percentage when compared to the 25–33% of enzymes in the proteome from bacteria and archaea. Apparently as a parasite, plasmodia dedicate fewer genes to enzymatic activity. In the erythrocyte, *P. falciparum* relies principally on anaerobic glycolysis for energy production. NAD$^+$ is regenerated by conversion of pyruvate to lactate. A complete enzyme set for the TCA cycle was identified, but it is not used for energy production via the respiratory chain as critical components of the ATP synthase are lacking. The TCA cycle probably functions as a supplier of intermediates for other pathways. The genome sequence predicts that the parasite can synthesize pyrimidines de novo, while it is incapable of de novo purine synthesis, which must therefore be imported. Likewise the parasite lacks the enzymes for amino acid biosynthesis. Amino acids are obtained by salvage from the host and by globin digestion. The trophozoite uses hemoglobin from the erythrocyte cytoplasm as an important food source. In the food vacuole the globin is hydrolyzed to small peptides, which then provide the amino acids needed by the parasite. The parasite synthesizes its own heme de novo. An important metabolic activity is the degradation of the heme moiety of the hemoglobin into hemazoin—large amounts of heme are toxic to the parasite.

**Drug Targets**

This heme degradation pathway is actually the target of classical drugs against malaria, namely quinine and especially chloroquine. The sobering fact is that despite all research on malaria, the impact of malaria in Africa has been increasing over the last decade mainly because of the slow, but constant advance of chloroquine-resistant malaria (Arrow et al. 2005). Fortunately, there is a Chinese herbal drug emerging that is currently the treatment of choice:
artemisinin from the plant *Artemisia annua*. This is now the showcase where a drug is cultivated by farmers on the field. Notably, the hunger of the parasite for hemoglobin is the target for this new drug: artemisinin also interferes with the degradation pathway of the heme.

**Plasmodium Proteomics**

However, there are clear limitations to the use of genomics in malaria research: only 35% of the predicted proteins have an identifiable function. Alternative approaches are thus needed to get into the secrets of this elusive pathogen. Large-scale mass spectrometry allowed a detailed proteome analysis. One study identified 1,300 (Lasonder et al. 2002), another even 2,400 proteins (Florens et al. 2002). The proteins were associated with different developmental stages, which allowed a first attribution of many hypothetical proteins to different stages. A complication was the observation of a substantial overlap of proteins found in different stages. For example, the antigenically variant proteins of the *var* and *rif* genes are not only found on the surface of infected erythrocytes, but were unexpectedly also expressed in sporozoites (Florens et al. 2002). These proteins are exported to the surface of the infected erythrocyte, where they mediate adherence of the infected red blood cells to endothelial receptors on the host blood vessels. These proteins represent important virulence factors, but owing to their location, they are also very exposed to the attack of the immune system. If the infected cell is destroyed before the parasite has completed its life cycle in the red blood cell, the infection chain will be interrupted. Immune evasion is thus an important task for the parasite. It achieves this task by having 59 *var* and 149 *rif* genes that can be expressed alternatively. The crucial role of the *var* genes is highlighted by their chromosomal position. In 24 of the 28 chromosome ends, the *var* genes are the first transcriptional unit. This telomeric organization is consistent with the exchange between chromosome ends as a means of immune evasion (Gardner et al. 2002).

**Transcriptome**

The next step providing new insights was the mRNA transcription studies on microarrays (Le Roch et al. 2003). Notably, 88% of the predicted genes were expressed in at least one stage of the life cycle. Half of the expressed genes were constitutively expressed identifying them as housekeeping proteins. Others were expressed in a cell-cycle-dependent way, which led to the definition of 15 expression clusters allowing tentative attributions of functions by the guilty through association principle. Interestingly, from *var* genes found at the chromosome ends (subtelomeric *var*) all but one showed low, constitutive expression and only one of them showed differential expression. Some *var* genes are located toward the centromere (“middle”part) of the chromosomes and they showed even more highly regulated expression (40- and 150-fold increases over basal level in sporozoite and trophozoite, respectively).
Organizing the Membranes

Apparently, you have to work hard to get a free meal from our erythrocytes. Some of the problems are self-created. The parasite contains many intracellular membrane systems and is also surrounded by a plasma membrane like any eukaryotic cell. In addition, within the erythrocyte it sits in a so-called parasitophorous vacuole, which is bound by a membrane; all are then wrapped with the erythrocyte membrane. This Russian doll construction of membrane systems gives you protection and creates new metabolic compartments to conduct parasite-specific metabolism. However, these multiple membranes have to be crossed by the substrates and end products of metabolism necessitating many protein transporters. Researchers had identified multiple tags at the N terminus of proteins destined for export. As a first ticket, a signal sequence recruited the protein into the secretory pathway within the parasite—a well-known process from other organisms. However, when this signal was cleaved, a second ticket became visible in some proteins that got them still further, the vacuolar transport signal. The barcode destined for further transport by the translocon in the vacuolar membrane was now deciphered (Marti et al. 2004; Hiller et al. 2004). As we live in the era of the -omes (genomes, transcriptome, proteome), researchers dubbed the ensemble of these exported proteins the malaria secretome (Przyborski and Lanzer 2004). The sheer size of the malaria secretome surprised even experienced malaria researchers: 320–400 proteins showed this translocon ticket. In fact, the malarial parasite rebuilds its home and directs the erythrocytes to its own nutritional and developmental needs.

Nutrient Import

As I am here concerned with the quest for food, I will focus in the finishing paragraph on one of these nutrient transporters. To put it into perspective: it takes a mere 48 h for the parasite to grow from an infecting merozoite to a huge trophozoite that gives rise to 20–40 new parasites. To fuel this enormous growth, the parasite needs nutrients, but the erythrocyte was not built to satisfy this appetite. The nutrients available in the uninfected erythrocyte simply could not sustain this need for food molecules (Kirk 2000). Hence the parasite had to target transporters also to the erythrocyte membrane. Not surprisingly, trophozoite-infected erythrocytes exhibited a 150-fold higher conductance than uninfected cells. The current was mainly carried by anions and abruptly abolished by channel blockers. Patch-clamp techniques identified a small ion channel present in about 1,000 copies per infected cell, which transports anions, sugars, purines, amino acids, and organic cations (Desai et al. 2000). This channel is probably also used to export metabolic waste like the enormous amount of lactate produced by the parasite.

From Cucania to Schlaraffia

With this transporter in place the parasite has unlimited access to small nutrients carried in the serum of its victim: the table is ready and as long as the host
survives this hard ride, the parasite lives in Schlaraffia. This imaginative country
became popular with a fairy tale by Bechstein, a follower of the Grimm brothers:
it is the country where milk and honey flows, sausages grow instead of fences
around the houses, grilled pigeons are flying into the mouth of the gourmands.
Here laziness is a virtue and diligence a vice. The story does not lack the fountain
of youth where you can exchange your aging better half against a young one.
The name of the land derives from the older German “slur” (lazy fellow) and
“affe” (monkey, here a stupid person who believes the story). Of course, you
must ask a mute for the way. Schlaraffia is not in Germany, clerics of the Dark
Ages fabled of the land Cucania, the Latin poet Lucian knew it, and the promised
land of Moses carries traits of Schlaraffia. You can categorize this story as one
of the endless myths on the lost paradise. For a biologist the story is nevertheless
interesting: it symbolizes the revolt of man against the world as it is. It is a
reverted world. Natural laws are reversed: organisms are growing younger and
humans are freed from the laws of thermodynamics imposing the quest for food.
To be precise and interestingly for this story, it eliminates only the quest part
for food, not the food itself. Eating as such is seen as a divine pleasure, only in
this dream it comes cost free. The French are the most consequent Schlaraffians
with their proverb: qui dort dîne (who sleeps, eats). Recall that the ancients
imagined their gods still eating nectar and ambrosia. Scholars do not agree on
what Homer meant by ambrosia except that it was a food needed to confirm the
immortality of the Greek gods and has a strong fragrant character. Even the
God of the Old Testament smells with satisfaction the food, which is burned in
his honor.

Let’s get back to biology: the dream of the promised land is only of short
duration for the malarial parasite—the host is not well, loses appetite, burns
food calories excessively in fever bouts, and might die eventually. If the parasite
does not want to die with its victim it must get to the next host. You have heard
that this is not an easy road for plasmodia, necessitating the transition through
mosquitoes. Did other organisms find the land where milk and honey flows?

Going for our Gut

The Land Where Milk and Honey Flows

Complexity of the Gut Microbiota

It is not only man dreaming of Schlaraffia, a land that is surrounded by a wall of
gruel, and to get there you have to eat your way through. Many organisms have
this dream and when considered superficially, the microbial inhabitants of our
gut come close to this goal. The gut microbiota is the unseen majority sitting in
us. It has been calculated that our commensal microbial flora exceeds our body
cells by a factor of ten with respect to cell numbers. On a cell basis we are thus
90% bacteria and only 10% human. Of course, bacterial cells are much smaller
than human cells, but they still contribute the appreciable amount of 1.5 kg to our
body weight. Most bacteria reside in the alimentary tract, the highest numbers are reached in the colon with an excess of $10^{11}$ bacteria per ml of colonic material. This population also exhibits a high turnover when considering that up to a half of the 120 g of feces, which we produce per day, is bacterial biomass, live or dead. Living in the gut of somebody else is a priori the closest you can get to Schlaraffia in biology. You get the food from what your host is eating. From what we learned in our survey of the quest for food, one should distrust this idyll and suspect that life is not as easy as one thinks.

Complementary Approaches

To every place where a table with food is ready, consumers will arrive. There is probably no place on the earth which fulfills the basic physicochemical requirements for life that is not colonized by organisms. If one organism has discovered a food source, more or less related fellow organisms are not far. This also applies to the gut. Researchers around D. Relman from Stanford University recently examined the intestinal “microbiome” of just three healthy adult subjects (Eckburg et al. 2005). Many studies like this were conducted before; what sets this study apart is the fact that they did not restrict their search to fecal material, but extended their sampling to six different anatomical sites of the colon. In addition, they sequenced more than 10,000 ribosomal DNA samples and obtained bacterial and archaeal sequences in the ratio of 10:1. In this sequence set they identified 400 bacterial phylotypes; the majority were novel sequences from organisms that cannot yet be cultivated. Most of the inferred organisms were members of the Firmicutes (e.g., *Clostridium*) and Bacteroidetes phyla. Proteobacteria to which the famous gut bacterium *E. coli* belongs yielded only few sequences, which was not so surprising as *E. coli* represents only 0.1% of the bacteria in the colon (Figure 7.6). So if there is Schlaraffia, it is so densely populated by so diverse prokaryotes that the free meal idea becomes a pure illusion. This dense and varied bacterial population is also a challenge for the researcher. Efforts to cultivate these bacteria or to describe their genetic variety as a function of the host species, host genetics, age, sex, geography, health, and disease becomes a Herculean task, if not a chapter from the myth of Sisyphus. Research on this task uses three different approaches:

1. Despite the relatively hopeless situation, many research papers describe just the number of different species by culture-dependent and culture-independent methods.
2. Other researchers put their hope into the so-called second Human Genome Project where the composition of the gut microbiota will be described by a systematic comparative metagenomics approach (Relman and Falkow 2001). With the rapid development of sequencing technologies and the ameliorating computational capacities to assemble prokaryotic genomes from shotgun sequencing, this approach does not lack attraction. It could reveal to us 2–4 million genes that are associated with the aggregated genome of the about
1,000 bacterial species that make up the microbiome and which indirectly add to our metabolic capacities. However, it is not a trivial task to reconstruct a biological reality from \textit{in silico} data.

3. Therefore, one could quote here a third school perhaps best represented by J. Gordon’s group in St Louis, who address the complexity, at first glance, by two paradoxical approaches. At one side they use a variety of experimental techniques combining the available -omics techniques with physiological experiments in animals conducted with the approaches of many different biological disciplines. On the other side, they simplified the experimental system dramatically by working in most of their studies with germ-free mice that were monoinoculated with a single bacterial species.

Control the Access

\textit{One might also approach the complexity of host-microbe interaction in the gut by applying first principle arguments. You might argue that the host does not appreciate the idea too much to share its food with uninvited guests. It will in all probability restrict the growth of its commensals. The control will not be easy. Anatomically, the gut lumen is, despite its difficult access for the physician and experimental researcher, not an internal part of the body—it is an external world. Even worse: it has to be filled regularly with food items that contain microbes. If you look at the human situation with the eyes of a bioengineer you might try to control the access of microbes to the gut in the first place.}
This is what human evolution actually did: relatively proximal in the alimentary tract it placed a highly acidic stomach. In a cyclic pattern, triggered by the meals, the pH of the gastric juice changes between highly acid conditions of pH values near 1 to values approaching relative neutrality (pH of 5). In parallel with this pH cycles the bacterial content in the stomach juice changes between $10^2$ and $10^4$ colonies per ml. Thus only low titers of bacteria enter the small intestine.

Developing Priorities

Next another engineering request is encountered: your own digestion priority should be put over the digestion by the commensals assuming that you have the enzymatic equipment to deal with the specific food item. For us, proteins are not too difficult to digest, neither are fats. However, our Achilles’ heel is the digestion of polysaccharides of plant origin. The major sites of nutrient absorption in humans are in decreasing order the duodenum, the jejunum, and the ileum. A bioengineer would thus design a bacteria-free zone over this region to avoid competition for nutrients, which we can digest and absorb. This is exactly what is observed: bacterial counts of the mucosa and lumen of the jejunum rarely exceed $10^4$ per ml. Here I have to specify that great geographical variations complicate the picture. Human populations from developing countries have a more substantial and complex microbiota in the jejunum: viable counts reach up to $10^8$ per g for the luminal content and $10^6$ for the epithelia-associated fraction. In contrast to the contaminated small bowel syndrome known from patients in industrialized countries, who show steatorrhoea (increased fecal fat) and vitamin B12 deficiency, a high bacterial load in the small intestine is widely distributed in Asian populations. However, while being common, it might not be without consequence: the jejunal mucosa in people from the developing world is frequently flat and shows leaf-like villi. It is currently unknown whether this condition impairs the absorption of nutrients and contributes to the undernutrition in these regions. A complicating factor in populations from developing countries might already be the high prevalence of subjects producing only small amounts of hydrochloric acid in the stomach. This impaired acid production makes these people not only more susceptible to cholera (a subject producing normal acid levels needs $10^4$ *Vibrio cholerae* to get infected, while the infectious dose is lower for low acid producers). It also has a lower capacity to sterilize the food material reaching the small intestine.

In the context of our armchair speculation along teleological principles we could postulate that the human host made a deal with the bacteria. It provides them a home in the colon, a region with a naturally flat mucosa endowed with only marginal absorptive capacity except for short-chain fatty acids. The latter is produced by the anaerobic polysaccharide-digesting commensal flora. These short-chain fatty acids can be absorbed and utilized by the host. In addition, it leaves them that part of the food which the human host cannot handle due to limitations of its enzymatic apparatus.
Nutrition of *Bacteroides*

From the diet, 8–18 g nonstarch polysaccharide, 8–40 g starch, 2–8 g oligosaccharides, 2–10 g unabsorbed sugars, 10–15 g protein and peptides, and 6–8 g fats reach the colon each day. This is a decent food filling for a fermentation vessel. As we will soon see, host components can be added to the food list of the gut bacteria. The colon is thus a great prospect for saccharolytic bacteria that have the necessary enzymatic capacities to deal with complex polysaccharides. You might take a glimpse into the contract signed between humans and *Bacteroides thetaiotaomicron* by reading the genome of the latter. *B. thetaiotaomicron* (it has even for microbiological standards a tongue-twisting name) is the numerically dominant gut microbe. It owns with 6.3 Mb a relatively large genome for bacteria. Fittingly, it encodes the greatest amount of polysaccharide-digesting enzymes of all sequenced prokaryotes. It comprises 172 glycosylhydrolases, which cover with their cleavage specificity a broad range of glycosidic bonds encountered in polysaccharides of plant origin (Xu and Gordon 2003; Xu, Bjursell et al. 2003). The next prolific carbohydrate digester of the human intestine is *Bifidobacterium* (Figure 7.7), the dominant gut microbiota of the breast-fed baby. The sequenced *B. longum* strain (Schell et al. 2002) contains 39 glycosylhydrolase genes, which is still remarkable when compared to the mere 18 glycosylhydrolase genes in *E. coli*. *B. thetaiotaomicron* has also outer membrane proteins, which bind starch to the bacterial cell surface. The bound starch is then hydrolyzed by an outer-membrane-bound α-amylase. The membrane location of these enzymes minimizes the diffusion of digested products and thus the cross-feeding of competitors. The oligosaccharide is then taken up by a porin into the periplasmic space where another α-amylase creates together with an α-glucosidase glucose monomers. Glucose is broken down in the cytoplasm to pyruvate via glycolysis; fermentation processes then create short-chain fatty acids (SCFA: acetate, propionate, butyrate in the ratio 70:20:10). In a European diet, about 50 g of carbohydrate is typically fermented per day, yielding 0.5 mol of SCFA with a total energy value of 150 kcal—the energy equivalent of one extra yogurt pot (Hooper et al. 2002). As SCFA are directly taken up by the colonic epithelium, they thus significantly contribute to the energy supply in man (McNeil 1984). The cooperation contract is thus honored from both sides and one could speak of a relation of mutual benefit, a symbiosis.

Has *Bacteroides* “Domesticated” Humans?

In a series of fascinating papers, the Gordon lab has extended this host–microbial relationship in the intestine to unexpected areas. The researchers colonized germ-free mice with *B. thetaiotaomicron* and followed the global transcriptional responses of the intestine to bacterial colonization using microarray technique (Hooper et al. 2001). The expressions of activators for digestive enzymes (colipase), transporters of glucose into the gut epithelium, and immunoglobulin transport out of the gut epithelial cell were increased, while
Bifidobacteria are nonmotile, nonsporing rods of varied shapes that frequently display V-shaped terminal clubs. They belong to the high GC content Gram-positive bacteria and are a pioneer colonizer of the human intestinal tract particularly in breast-fed babies. They lack a pathogenic potential and are thus the classical commensal flora of the gut not only of warm-blooded vertebrates, but also of some insects.

Figure 7.7. Bifidobacteria are nonmotile, nonsporing rods of varied shapes that frequently display V-shaped terminal clubs. They belong to the high GC content Gram-positive bacteria and are a pioneer colonizer of the human intestinal tract particularly in breast-fed babies. They lack a pathogenic potential and are thus the classical commensal flora of the gut not only of warm-blooded vertebrates, but also of some insects.

lactase expression decreased. Spectacular was the upregulation of a protein involved in epithelial barrier function. Notable was also the increased expression of angiogenin-3, which plays a role in the growth of blood vessels. Indeed, it was observed that germ-free mice did not complete the normal postnatal gut development. The mice had arrested capillary network development. The colonization with *B. thetaiotaomicron* induced this developmental program and the maturation of the intestinal capillary system was completed within 10 days (Stappenbeck et al. 2002). *B. thetaiotaomicron* also induced the production of antimicrobial peptides from Paneth cells in the bottom of the crypts from the gut epithelium. In vitro activity was demonstrated against competing bacteria including pathogens. Not enough with that, the introduction of a microbial flora to germ-free mice produced 60% increase in body fat content despite reduced food intake. A circulating lipoprotein lipase inhibitor is suppressed by *Bacteroides* leading to a greater deposition of triglycerides in fat cells. The gut microbiota
could thus affect the energy harvest from the diet and energy storage in the body (Bäckhed et al. 2004). The researchers extended their investigations recently to mutant mice strains that were genetically disposed to obesity (ob/ob mice), which showed concomitantly a shift in the microbiota from Bacteroidetes to Firmicutes (Ley et al. 2005). The murine host and its gut microbiota thus showed unexpected interactions and cross-talks speaking for a finely tuned coevolution between these partners over millions of years.

Double Strategy: Fucose Versus Glycan

I will illustrate this relationship by mentioning a mouse enzyme that contributes food to *B. thetaiotaomicron* in the gut. The enzyme in question is α 1,2-fucosyltransferase, whose expression in the small intestinal epithelium is induced by *B. thetaiotaomicron*. This enzyme adds a terminal fucose pentose sugar to glycoconjugates in the gut mucosa (Bry et al. 1996). Where is the benefit for the gut microbiota here? The answer is straightforward: the mammalian host sacrifices a substantial amount of biological material to its gut microbiota. This host-derived bacterial food comprises 5–8 g secreted digestive enzymes, 3 g IgA antibodies, 2–3 g mucins and 20–30 g desquamated epithelial cells per day. This is a substantial extra amount of food if you learned to use it. The genome sequence of *B. thetaiotaomicron* is a “read my lips” recipe: *B. thetaiotaomicron* has a fucose-utilization cluster, which enables it to live from fucose residues cleaved off from host glycoconjugates. The *fuc* operon is preceded by a repressor gene. FucR blocks the transcription of this operon unless fucose is present, which prevents the inhibitory action of the repressor. When the fucose concentration decreases and *B. thetaiotaomicron* loses this valuable carbon source, another function of fucose becomes apparent. Fucose is a co-repressor of another locus called *csp* for control of signal production. This signal molecule, whose molecular nature is not yet defined, will induce the murine α 1,2-fucosyltransferase. The gut bacterium convinces its host to fucosylate its glycoconjugates to provide food for its commensal (Hooper et al. 1999). In this way *B. thetaiotaomicron* has the flexibility to change from diet-derived polysaccharide to a host-derived glycan foraging. This double strategy makes the gut commensal relatively independent of variations in the dietary food supply. When the diet is the food source, *B. thetaiotaomicron* assembles on food particles and outer-membrane polysaccharide-binding proteins and glycoside hydrolases are induced. The metabolic reconstruction of RNA microarray data is consistent with the delivery of the hexoses mannose, galactose, and glucose to the glycolytic pathway, and the pentoses arabinose and xylose are funneled into the pentose phosphate pathway. If mice were deprived of sugars in the diet, *B. thetaiotaomicron* changes to an endogenous (= host-derived) source of glycans in the cecum. In accordance with the different chemical structure of this food source, sialidase, hexosaminidases, mucin-desulfating sulfatases, and chondroitin lyases are induced (Sonnenburg et al. 2005).
Value and Limitations of the Reductionist Approach

A number of studies illustrate now the human–Bacteroides symbiosis, where the human host provides shelter and food and the bacterium offers contributions to the postnatal gut development, host physiology, and nutrition. This view culminates in a review entitled “Honor Thy Symbiont” (Xu and Gordon 2003). We should not forget, however, that the data were obtained with the reductionist approach. This approach has a long tradition in biology and is one of the most powerful approaches in structuring biological complexity. Yet it comes at a price, namely that it describes only a 1-D picture of the complexity surrounding us. While being in the tradition of Max Delbrueck, one should not forget that current biological model systems reduce the biological complexity to systems like phage lambda interacting with E. coli K-12 in a test tube or on an agar plate. The consequence of the reductionist approach in the case of phage lambda was that on the one hand it became one of the best-studied biological systems, but on the other hand we do not have a single study that describes the interaction of a coliphage with E. coli in its natural niche, the gut, in any molecular detail. This lack of data now renders phage therapy approaches of E. coli diarrhea with orally applied phages rather difficult (Figure 7.8).

If I now transfer this argument to the gut microbiota, we see currently a strong trend for the development of probiotic, i.e., health-promoting, bacteria. Interestingly, the majority of the approaches were conducted with gut bacteria belonging to the genera Lactobacillus and Bifidobacterium. Some like the Lactobacillus rhamnosus strain GG showed beneficial health effects in a number of carefully controlled clinical trials. However, the genome of this strain has not yet been published, consequently -omics techniques were not yet applied to this

![Figure 7.8](image_url)

**Figure 7.8.** A collection of T4-like phages that target pathogenic E. coli strains, which are developed in the laboratory of the author to test the phage therapy concept against E. coli diarrhea as proposed by the codiscoverer of phages. Panel F shows T4 phages in various stages of injection of their DNA genome into a remnant of an E. coli cell.
strain and its interaction with the host and competing gut microbes has not yet been described to any greater molecular extent. We can thus only relatively vaguely define the probiotic properties of such strains.

Therefore, we definitively need both fields to reach a common ground, meaning that the Bacteroides work reaches out for more realistic experimental studies (not only strain combinations, but also probiotic clinical trials), whereas the Lactobacillus and Bifidobacterium work extends from black box approaches in clinical trials to mechanistic and molecular approaches to elucidate the basis of probiotic properties.

The Thin Line Between Symbiont and Pathogen

Bacteroides fragilis

So far we got the impression that B. thetaiotaomicron is a versatile gut bacterium that has grown to intestinal dominance because it learned in its evolution a number of lessons which led to a superior adaptation in its niche. Mice and B. thetaiotaomicron exchange nutrients for mutual benefit. In fact, bacterial signals have become important cues for the maturation of the intestine and important regulators of host physiology. All these observations point to a symbiotic relationship (both partners benefit) and not just a commensal relationship (neither is harmed). This adaptive success is reflected by the fact that the genus Bacteroides accounts for about 30% of the fecal isolates. We have seen the Greek god Proteus several times in the current book and he lifts his head again. However, even if B. thetaiotaomicron numerically dominates this fraction, it is not the only Bacteroides species in our gut. The cell numbers of B. fragilis is 10- to 100-fold lower than those of B. thetaiotaomicron. These bacteria are probably not brothers, but more distant cousins. The differences between two bacterial species can be deceivingly large. Even within the confines of a single bacterial species, different isolates can differ by as much as 15% in gene content (e.g., Salama et al. 2000 for the stomach pathogen Helicobacter pylori), which is enormous when considering that the application of this microbial standard would require to place all primates into a single species. Humans and chimpanzees are, for example, 98.7% identical in their genomic DNA sequence (Enard et al. 2002). This broad bacterial species definition practised by current taxonomists also explains why we have, at the moment of writing, less than 8,000 recognized bacterial species (http://www.bacterio.cict.fr).

Capsular Polysaccharide Variation

When analyzing B. fragilis a different picture emerged. B. fragilis is covered with multiple polysaccharides. The structure of two polysaccharides, PSA (polysaccharide A) and PSB, was elucidated and consists of repeating units containing a terminal α 1,2-fucose moiety. The bacterium proteome contains an enzyme that handles fucose, the biochemical details are somewhat complicated, but the essential feature is that this bacterial enzyme looks like a fusion of two mammalian enzymes handling fucose. Not surprisingly, B. fragilis can
take up fucose from the medium and incorporates it with the help of this hybrid enzyme into capsular polysaccharides and glycoproteins (Coyne et al. 2005). Glycoproteins are rare in bacteria. The authors of this article concluded that the bacterium used a host-like pathway for bacterial surface fucosylation to serve as a molecular disguise for *B. fragilis* against the immune system of the host. *B. thetaiotaomicron* also possesses this gene, but fucose is apparently more used as food than for molecular mimicry as also reflected by the lower number of capsular polysaccharide genes. It thus seems that both species derive from a common ancestor, which split into two ecological lines despite the fact that both bacteria still sit in the same niche. However, the mammalian gut has many niches. Somewhat simplified *B. thetaiotaomicron* occupies the lumen, whereas *B. fragilis* targets the mucosal surface. This specialization within the same ecological setting necessitated genetic adaptations, which can still nicely be read from the blueprint of the two bacterial genomes (Kuwahara et al. 2004).

Two Different Lifestyles

Both bacterial species cope well in their environment essentially by deploying two strategies: first, the exceptional capability to use a wide range of dietary polysaccharides due to gene amplification and second, the capacity to create variable surface antigens with capsular polysaccharides. *B. thetaiotaomicron* excels in the first strategy, while *B. fragilis* is a master in the second. The absolute number of capsular polysaccharide clusters is not so different between both species: nine versus seven. The genomes differ substantially in genome size: 6.2 vs. 5.2 Mb. While *B. fragilis* is smaller, it excels in flexibility. The Comstock lab in Boston documented its genomic dynamics. They raised antibodies against the wild-type strain expressing the different capsular polysaccharides. Then they created mutants, which inactivated the expression of a single polysaccharide cluster. When the antiserum against the wild-type strain was now absorbed with the mutant strain, it pulled out all antibodies against the shared polysaccharides, and only antibodies against the mutated polysaccharide remained in solution. In this way the investigators obtained monospecific sera. To their surprise these sera labeled, in electron microscopy and flow cytometry, only a portion of any given culture. Positive and negative colonies split again into both phenotypes defining a reversible on–off phenotype. Genetic analysis revealed the reversible inversion of a DNA segment ahead of the polysaccharide synthesis cluster. This operation brings the promoter in either the on or the off position (Krinos et al. 2001). The assembly process of the *B. fragilis* genome was actually complicated by the extensive amount of DNA inversions that occurred even in the bacterial colony from which the DNA was extracted for sequencing (Cerdeno-Tarraga et al. 2005). The DNA inversions control variable gene expression and the genome researchers from the Sanger Center distinguished several types: On one side, there is recombination across inverted repeats that flank the promoter. They also identified 30 different enzymes, which resembled site-specific DNA recombinases (“invertases”). One enzyme, the Mpi recombinase, apparently plays
the master regulator for the switches ahead of the capsular polysaccharide clusters (Coyne et al. 2003). *B. thetaiotaomicron* has also these genetic elements, but it seems to use it less frequently than *B. fragilis*. On the other side, *B. fragilis* also has intergenic shufflons, where gene segments coding for protein domains are exchanged, creating proteins with new biochemical properties.

Pathogenic and Probiotic Properties

This phase variation is an essential part of the success for the colonization of gut epithelia by *B. fragilis*. We pay for this evolutionary success: *B. fragilis* is the major anaerobic Gram-negative bacterium isolated from abscesses, soft tissue infections, and bacteremia that arise by contamination from the gut. A number of comparisons are instructive here: *B. fragilis* accounts for between 4 and 13% of the normal human fecal microbiota, but is present in 63–80% of human *Bacteroides* infections; *B. thetaiotaomicron* accounts for between 15 and 29% of the fecal microbiota, but is associated with only 13–17% of infection cases (Cerdeno-Tarraga et al. 2005). Except for two hemolysin-like genes, the two strains do not differ in virulence-related genes if one does not account for the supplementary capsular polysaccharide genes and their regulation. *B. thetaiotaomicron* has thus clinically too much residual potential for pathogenicity to qualify as a health-promoting, probiotic strain. The food industry screens for members of the natural human microbiota that have health-promoting properties. *Lead strains come from the genera Lactobacillus and Bifidobacterium*. The credential of the first is its long safe use in food fermentation and of the second is its dominance in the gut microbiota of the breast-fed baby. The idea of probiotic bacteria is not new and goes back to Metchnikoff, a pioneer medical researcher who propagated the idea to modify our gut microbiota by alimentary means to achieve health benefits. That this idea is not just part of a “green” health folklore is demonstrated by clinical tests that objectively demonstrated measurable health effects after oral application of a gut-derived *Lactobacillus* strain (Sarker et al. 2005). Changing the composition of our gut microbiota might be an innovative approach when addressing nutritional problems such as the current obesity epidemic (Ley et al. 2005).

Antibiotic use in Animal Rearing

In the agricultural context, this approach is already a long-established, although currently hotly debated, practice. To shift the ratio of the gut bacteria by different diets might be difficult when realizing the nutritional flexibility of *B. thetaiotaomicron* (Sonnenburg et al. 2005). In animal husbandry, antibiotics are added to the feed. This intervention significantly increases the weight gain of the animals. As the quantities of antibiotics used are in the subtherapeutical dose range, it seems unlikely that the extra weight increase is gained through a reduced incidence of infectious disease. This idea is not far-fetched because in developing countries nutritionists documented that repeated episodes of infectious diseases, especially of diarrhea, cause a measurable flattening of the weight increase.
Malnutrition in these countries is the combined effect of insufficient food intake and diarrhea. The alternative interpretation for the effect of antibiotics in animal feed is a shift in the composition of the gut microbiota with a concomitant increase in the efficiency of conversion of fuel in the diet to body mass. If this hypothesis should be verified it would question the symbiotic character of the established gut microbiota as it would be responsible for a drain of food energy. In contrast to a substantial literature, which investigates the effect of antibiotics feeding on the relentless rise of antibiotics-resistance genes in bacterial pathogens, not much is known on the nutritional effects of antibiotics (Gustafson and Bowen 1997). Surprisingly, the jury is still out in this eminently important practical question.

**Immune Response to Commensals**

Even if the opinion of researchers on the role of specific bacteria in the gut microbiota still remains split, there is an objective mean to assess their role. You simply ask the immune system of the host. Robert Koch has established microbial rules to establish a link between a bacterium and a given disease. Chance isolation of pathogenic bacteria from healthy subjects is commonplace in diarrhea epidemiology. A common corollary in infectious disease medicine is to ask whether the patient responded to the isolated bacterium immunologically. Again: the host defenses against the very heavy load of intestinal commensal bacteria are poorly understood. This is surprising as >70% of the daily produced antibodies drain down the gut (Macpherson and Uhr 2004) and are quickly lost with the feces. Why should the body dedicate so much synthetic activity to secretory antibodies that have such a short half-life compared to the circulating antibodies, which have half-lives measured in months? The answer of an evolutionary biologist would probably be this: because they fulfill an important role to protect the body against microbial attack coming from the gut. This seems plausible, but what are the target organisms? The secretory immune system is not particularly efficient against enteric pathogens—repeated episodes of diarrhea traced to the same enteropathogenic species are well known. Also the researchers trying to develop vaccines against common gut pathogens like *E. coli* and *V. cholerae* suffered a lot from the short-lasting immune response to these antigens. Apparently, the gut immune system does not have a good immunological memory. The gap in this knowledge was recently filled with important data from the University of Zurich (Macpherson et al. 2000). The researchers reported that—in contrast to conventional views—a large proportion of the intestinal IgA is directed against cell wall antigens and proteins of commensals. These antibodies are specifically induced in response to the presence of commensals like *Enterobacter cloacae*, the predominant commensal in the mice of the Zurich lab. Notably the antibody response is independent of T cells and the authors speculated that their data shed light on a primitive, specific, antibody-dependent immune system that developed in the gut as a defense line against the gut microbes lurking “ante portas.” The system is quite sophisticated: intestinal dendritic cells located in the mucosa take up and retain small numbers of live commensals. These cells are apparently
on a spying mission to induce the selective IgA response needed to protect the mucosa against penetration by commensals (Macpherson and Uhr 2004).

Intestinal Desquamation and Peristalsis

In this context, I have another subject that puzzled me. What is actually the evolutionary reason for the high rate of renewal of the intestinal epithelia? Approximately, every 3 days the entire intestinal mucosal epithelia are replaced. As the gut was designed by evolution for surface amplification, the number of cells desquamated is enormous and represents a substantial metabolic load for the body. Is it imaginable that the desquamation is a defense process against gut bacteria that succeeded to get a foothold on the epithelia? Before they could do harm, they are removed by the exfoliation of the villi? This suspicion was recently confirmed by the reaction of mice experimentally infected with the nematode (Figure 7.9) *Trichuris trichura*, the 4-cm-long whipworm. Worldwide, about 800 million people are infected with this parasite, which sticks with its anterior whip to the superficial mucosa of the cecum and ascending colon, each worm sucking 0.005 ml blood per day. This amount is so small that infections remain normally asymptomatic. Only heavily infected people suffer mild anemia and bloody diarrhea. However, one should not underestimate the worm burden: the life expectancy of the adult worm is 1 year and a single female produces 5,000 eggs per day. The eggs are transmitted to new hosts by fecal contamination. In countries with low hygiene level, 75% of school children are thus infected with this worm. It is thus clearly better not to have these uninvited guests. Mice strains differ in their susceptibility to the worm and it was revealing to compare susceptible with resistant strains. In the susceptible mice, a significant crypt hyperplasia was observed, while the resistant mice showed a greater loss of cells from this proliferative compartment. In the latter, the cells moved at a much faster rate along the crypt–villus axis. The crypt contains a pool of

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**Figure 7.9.** Parasitic worms. Four Nematoda worms (Phylum Aschelminthes, roundworms): 1. *Ascaris lumbricoides* (*a*, rear; *b* and *c*, front-end views of the worm). The durable egg (*d*) is ingested with contaminated vegetables and fruits, the animal hatches in the intestine, travels from there into the lungs, then back into the mouth and arrives after swallowing in the feces to begin a new life cycle. 2. *Trichinella spiralis* causes a food-borne disease when uncooked muscle meat containing encysted worms is eaten (*a*, female; *b*, male worm; *c*, young larvae). The worm reproduces in the intestine and the larvae then invade the muscle fibres where they grow; the center, bottom shows free larvae in the muscle (right) and encysted larvae (left). Panel 7 shows a closely related, common, but relatively harmless human parasite *Trichocephalus dispar* (*a*, egg; *b*, female; *c*, male invading with its body the gut mucosa). *Anguina tritici* (5) is a plant parasite; the worm infects wheat, the grain becomes filled with worm larvae. The fluke *Fasciola hepatica* (6, class Trematoda) belongs to the worm phylum Plathelminthes (flatworm). It causes the liver rot disease in sheep. The adult animal shows at the top a muscular sucker with which it attaches to the host. Panel 8 shows a representative of the Nemertini class of flatworms, the depicted animal is a freshwater inhabitant.
stem cells, which divide and give rise to enterocytes, enteroendocrine cells, and goblet cells that migrate in vertical bands to the top of the villi. The enterocyte differentiates during this journey into a mature absorptive cell, the goblet cells secrete the mucus, and the enteroendocrines sense the presence of the nutrients and respond with the release of hormones. These cells reach within 5 days the exclusion zone at the tip of the villus, from which they are shed into the lumen at a rate of $10^{11}$ cells per day in humans. When exposed to the whipworm, the resistant mice increase the cellular turnover and drive the “epithelial escalator” to expel *Trichuris* (Cliffe et al. 2005). As one could expect, the interplay between worm and host is more complicated because the escalator is in addition under immune control. The worm has apparently learned to induce one cytokine that favors crypt cell hyperplasia creating a neat niche and another that slows the escalator. On the other hand, the goblet cells secrete a substance that disorients the chemosensory apparatus of the nematode, leading to the loss of its foothold (Artis et al. 2004).

*Peristalsis is partly explained by the need to push the nutrient bolus through the intestine, while at the same time it serves to flush the gut lumen. Luminal bacteria, which do not divide more rapidly than the rate of peristalsis, will not increase in number. Some enteropathogenic bacteria have a high replication rate in the small intestine as indicated by their high numbers in the stool (e.g., $10^8$ cfu = colony forming units V. cholerae/ml cholera stool). The increased peristalsis observed in diarrhea patients might be an adaptive response. The host increases the flushing to get rid of the pathogen by expulsion. Therefore medication to decrease the peristalsis (e.g., loperamide) is regarded by some clinicians as counterproductive. If you consider further that—as mentioned before—the majority of the antibodies synthesized by the body are secreted into the gut, it could appear that the body builds a high firewall against unfriendly intrusions by its gut microbiota. Even gut bacteria that enjoy a GRAS (generally regarded as safe) status like Lactobacilli are not a rare finding in blood samples sent to the clinical microbiologist. Even harmless gut bacteria—once in the circulation—might have a residual pathogenic potential. In the case of lactobacilli, subjects at risk of developing pathologies with lactobacilli are those with defects on the heart valves.*

Critical Mutations that Lead to Loss of Control over the Gut Microbiota

The analysis of a mutation in mice underlined the importance of secretory IgA as the front line defense in the gut mucosa. The mutation concerns an RNA editing enzyme called AID, a cytidine deaminase. The biochemical action of this enzyme is of lesser interest in our context. However, if the gene is defective, class switching from IgM to IgA-producing cells does not take place in the gut lamina propria, and somatic hypermutation of the IgA antibodies does not occur either. Therefore these mice secrete great amounts of IgM into the gut (Fagarasan et al. 2001). This immune defect has important consequences: the mice lose the control over the anaerobic microbial flora in the small intestine, which expands 100-fold. This increased antigenic load leads to a hyperplasia
of isolated lymphoid follicles in the gut, which is probably induced secondarily by the increased antigen load in the small intestine. In fact, antibiotic treatment of the AID−/− mice abolished the exaggerated immune reaction toward the gut microbiota (Fagarasan et al. 2002). When using culture-independent techniques such as 16S rRNA sequencing, these researchers discovered that the lion’s share of the increased microbiota was contributed by a group of bacteria, which they called SFB (segmented filamentous bacteria). These bacteria replaced the lactobacilli found in young and clostridia and Bacteroides found in older AID+/+ mice (Suzuki et al. 2004). SFB strongly attach to the gut epithelium, which was not prevented by the normal levels of secretion of antimicrobial proteins by the Paneth cells at the bottom of the crypts. When primed IgA B cells from normal mice were introduced into the circulation of the mutant mice, the SFB retreated to the lower segments of the intestine. Apparently, the local immune system controls where it wants to have the gut commensals.

Bacteroides and the Immune System

If we look at all these data: are the gut microbes friends or foes? What is the purpose of the immune response to these commensals? Does it want to restrict the microbes to the lower parts of the gut to limit nutritional competition? Or is its main purpose to prevent inappropriate immune activation of the gut mucosa by adhering microbes potentially leading to inflammation? Recent work showed that the gut microbiota is important for the development of the immune system. It was, for example, demonstrated that monoclonization of germ-free mice with B. fragilis is sufficient to correct immunological defects that occur in the absence of a bacterial microflora in the gut of axenic animals. The researchers identified polysaccharide A (PSA) as the bacterial component that directs the maturation of the developing immune system. T-helper cell imbalances and lymphoid organogenesis were corrected. Mechanistically, PSA activates CD4+ T cells via dendritic cells and elicits appropriate cytokine production (Mazmanian 2005).

To get more clarity on the role of Bacteroides it would be interesting to investigate the immune response against this gut bacterium. Researchers working with streptococci have already realized that there is anyway a thin line between commensals and pathogens (Gilmore and Ferretti 2003). The play with the variable expression of the capsular polysaccharides in B. fragilis is a well-known immune evasion strategy in pathogens. It is probably significant that the mucosa-associated B. fragilis plays these cards more actively than the more lumen-restricted B. thetaiotaomicron. Probably, Bacteroides is a bit of all: symbiont, commensal, and pathogen. With respect to the latter attribution: enterotoxigenic B. fragilis is a small, but significant contributor to diarrheal disease in developing countries (Pathela et al. 2005).

Coprophagy

We still lack a clear answer to many questions concerning the role of the gut microbiota for humans. Here I just want to mention a question that puzzled
me and which might be relevant in this context. If the stool is composed in its majority of bacteria, it would represent a valuable source of relatively easy to digest proteins in times of starvation. Yet except in a recent Russian film from northern Finland (Kukuschka) I have never come across reports that coprophagy was practised during famines. Humans have a strong aversion against their excrements, although this seems to develop only in early childhood and is thus not inborn. The gut is not a major organ for the elimination of metabolic waste when compared to urine. Only relatively few biochemical waste is excreted by the feces (e.g., the heme ring without the iron atom). The feces should thus not be too toxic from a chemical side. In support of this hypothesis, coprophagy, the eating of one’s own feces, is well known to laboratory biologists as mice and rabbits practice it in captivity. Primates are instructive in this respect. Primates have two principle strategies to place gut microbes. Microbial fermentation can occur in the enlarged foregut (stomach) as in langurs (Presbytis) and colobus monkeys (Colobus). They have large stomachs with many pouch-like sacs. Cecum-fermenting animals like the gorilla and the sportive lemur (Lepilemur) have simple stomachs, but a large, divided cecum. In the latter group food is digested in the stomach before microbial fermentation takes place. Simple carbohydrates from fruit and nectar are digested and absorbed in the proximal parts of the intestine, while the complex polysaccharides are handled in the distal parts by the gut microbes. The advantage of this strategy is that plant material is not well broken down in the upper parts where highly efficient absorptive epithelia are found—this means that plant toxins pass into the lower part of the gut and are thus mainly handled by the gut microflora. The microbes detoxify the plant material and spare the liver this work and the chemical insults. If toxins are freed unmodified, then a remedy is that the colon epithelium is less absorptive and the toxic load to the body is still kept low. The price you pay for this strategy is that your exploitation of the nutrients in the food is reduced. Gorillas have a simple answer to this dilemma, they ingest their own feces as observed in the wild. There is thus a second passage of food material through the intestine after detoxification of the plant material. Why do humans not practice coprophagy even in times of hunger? One could imagine that the microbial content of our feces poses a risk when recycled via a second round of ingestion. There might be a trade-off between diarrhea risk and extra nutrition. Cooking could, however, shift the infection risk ratio. I will stop this issue here, which will be as emotional as cannibalism — because of lack of data. In the scientific literature human coprophagy is exclusively reported in psychiatric patients.

Janus Faces: The Case of Vibrio cholerae

Human Categories do not Fit a Complex Reality

Bacteria do not have an obligation to fit into human categories like symbiont, commensal, or facultative pathogen. These categories were developed by scientists to mentally order their observations. The diversity in Nature perplexes
our minds more than it unsettles the organisms. I will illustrate the multifunctional ecological role of microbes with V. cholerae and its phages. In fact, extreme genomic diversity was described in coastal bacterioplankton populations (Thompson et al. 2005) and diversity might be a guarantee for biological stability. Evolution is the ultimate opportunist: why shouldn’t an organism profit from opportunities that are offered to it? It does not care about good and evil—categories, which got a sense in the biological world only after the arrival of self-conscious human beings, at best a few million years ago. Nature is autonomous and not obliged to respect our scientific terminology frequently coined with an eye on the impact of microbes on human life. Indeed, there might be an uninterrupted continuum from symbionts to pathogens and some organisms have several roles in the natural order, which obliges them to carry Janus faces when seen with our eyes. Janus is that double-faced Roman god who was in Roman liturgy invoked as the first of any gods and thus a good patron for biology.

**Vibrio cholerae** in the Environment

I will illustrate the Janus analogy with recent research papers dealing superficially with another gut bacterium, *V. cholerae*, the cause of cholera. This bacterium is such a dreaded pathogen in human history that its categorization in our human system of bacterial utility seems to be an easy task: it is a culprit. However, while not being innocent, it could plead guilty only by association with bad company. Slightly simplified, the following story can be told for *V. cholerae*: this bacterium can be found in many coastal waters in the tropics and subtropics. Its major habitat seems to be brackish waters and estuarine systems (Colwell 1996). This observation fits with the epidemiology of cholera—the disease originated in coastal areas such as the Bay of Bengal and in South America. The reason for this niche preference is probably the nutritional basis of this marine organism. *V. cholerae* produces a mucinase, an enzyme that degrades mucin. These compounds are encountered in the gut, hence its association with the human gut, but in the natural environment *V. cholerae* finds mucin-like substances in algae (*Volvox*) or in the mucilaginous sheath of the cyanobacterium *Anabaena*. *V. cholerae* also elaborates a chitinase and plays an important role in the remineralization of chitin in the ocean. We should thus not be surprised that *V. cholerae* growing on chitin induces the expression of a regulon consisting of 41 genes involved in chitin colonization, digestion, transport, and assimilation. Growth on chitin also induces in *V. cholerae* a type IV pilus that is in other bacteria associated with DNA uptake. Indeed, chitin thus induces natural competence in *V. cholerae* (Meibom et al. 2005). This observation has two facets. One is nutritional: extracellular DNA is present in tens
of micrograms per liter of seawater; this concentration is three to four orders of magnitude higher in sediments and biofilms. DNA at that concentration becomes an interesting source of carbon, energy, nitrogen, and phosphorus (Bartlett and Azam 2005). DNA, which is taken up by transformation, can in addition promote DNA repair and genetic diversity. This process could explain the mosaic structure of the \textit{V. cholerae} genome and the presence of the phage-encoded cholera toxin gene in cells that lack the receptor for the phages carrying this gene.

\textit{V. cholerae} was also found associated with egg masses of the nonbiting midge \textit{Chironomus}, which lays its eggs into fresh water ponds. The eggs form a row, are folded in loops, and are embedded in a thick gelatinous casing. The gelatin is consumed by \textit{V. cholerae} resulting in bacterial growth, while the released insect eggs mostly failed to hatch (Broza and Halpern 2001). So for \textit{Chironomus} and \textit{Anabaena}, \textit{V. cholerae} is a predator; for a copepod, it is a commensal. Rita Colewell speculated that the cholera toxin CT, which is responsible for the profuse diarrhea observed in cholera patients, helps in the osmoregulation of the copepod by facilitating the efflux of Na\(^+\) out of the gut when CT binds the CT receptor. The cholera toxin was therefore possibly designed for a different, perhaps even mutualistic, purpose before it became a potent toxin for humans. Part of the beneficial effect of CT can be still observed in humans: when given in small doses CT is one of the most potent adjuvant for eliciting a secretory immune response to mucosal antigens (Hajishengallis et al. 1995).

Context-dependent Toxins

This Janus-face of bacterial virulence factors is even better documented for a marine Vibrio, \textit{V. fischeri}. This Vibrio releases a tracheal cytotoxin, which acts in synergy with lipopolysaccharide (LPS) to trigger tissue development in its mutualistic symbiosis with the squid \textit{Euprymna scolopes}. The details of this process are of minor interest here: they concern the development of a light-emitting organ in the squid where the luminous bacterium offers its help. The toxin induces an inflow of squid hemocytes, followed by apoptosis (controlled cell death) induced by LPS resulting in epithelial regression. The toxin plays an important role as morphogen for this squid organ (Koropatnick et al. 2004). \textit{Bordetella pertussis}, the cause of whooping cough, and \textit{Neisseria gonorrhoea} elaborate a similar compound with comparable effects: the loss of ciliated cells from mammalian respiratory epithelia and the fallopian tube epithelia, respectively. \textit{However, this is destructive pathology, not destructive morphogenesis}. It is hard to tell whether this compound is a mutualistic morphogen or a toxin. \textit{The answer is context-dependent, a typical Janus face}. 

\textit{Evolution uses its inventions for many purposes}. One could argue that humans as evolutionary newcomers simply got in the way of microbes and suffer collateral damage as a mere evolutionary accident. \textit{However, humans became so abundant on the globe that microbes would miss an important ecological niche if they would not exploit this possibility}. Actually, microbes do not weigh their chances and choose on their survival strategies. \textit{The evolution game
selects simply those organisms that succeeded to multiply in the most efficient way, whatever the logic of their replication strategy.

Epidemic Cholera Strains

\textit{V. cholerae} has another fascinating Janus face. Epidemic cholera strains belong to just two O-serotypes determined by the chemical structure of the LPS decorating the bacterium: O1 and O139, the latter appeared only in 1992 (Faruque et al. 2003). Why are just two serotypes pathogenic from the about 200 O-serotypes described in environmental \textit{V. cholerae} strains? The answer to this paradox is the host range of a filamentous phage CTXΦ, which carries the cholera toxin genes ctx\(A,B\) (Waldor and Mekalanos 1996). It resembles in its genetic organization the classical filamentous \textit{E. coli} phage M13, the famous early cloning vector. This toxin-encoding phage recognizes a receptor on the susceptible \textit{V. cholerae} cell that is called the toxin-coregulated pilus (TCP). This structure is a fiber of the polymerized pilin protein (TcpA). TCP functions as an important virulence factor and is the main intestinal colonization factor of the pathogen. This virulence factor/phage receptor is not encoded by the core part of the \textit{V. cholerae} chromosome. Actually, here I must speak of chromosomes in plural as quite unusually for the sequenced bacteria, \textit{V. cholerae}’s genome consists of a large and a small chromosome of about 3 and 1 Mb in size (Heidelberg et al. 2000). The region encoding the TcpA protein belongs to a 40-kb-long mobile DNA element that was initially described as another filamentous phage, with TcpA being the major coat protein of this purported second filamentous phage (Karaolis et al. 1999). These data gave rise to wide-ranging models of how the cooperation between two phages created a pathogen. The phage nature of the genetic element was later questioned and it is probably better described as a pathogenicity island with phage-like properties. The current data suggest a model of a benign ancestor \textit{V. cholerae} strain O1, which acquired TCP by lateral gene transfer and was then infected by the temperate phage CTXΦ, which itself had somewhere acquired the ctx\(A,B\) genes, possibly by an incorrect excision event from a heterologous host. This sequence of events created the epidemic \textit{V. cholerae} O1 El Tor strain. Epidemic \textit{V. cholerae} strains show an unusual low degree of genetic diversity in microarray analysis with only 1% differences in gene content, suggesting a recently diverged lineage (Dziejman et al. 2002). The newly emerged epidemic \textit{V. cholerae} serotype O139 belongs to this lineage and has experienced a replacement of the O1- by an O139-specific LPS gene cluster, resulting in a serotype switch (Faruque et al. 2003).

Cooperation with a Phage

If I remain in anthropocentric view, \textit{V. cholerae} cannot just point to the CTXΦ phage as the culprit. It was not simply invaded by a parasitic mobile DNA element. There is a lot of evidence which points to a close collaboration between phage and bacterium in the pathogenic process. Just to mention some recent research literature: The bacterium takes care to integrate the phage as a prophage,
in some strains both in the large and the small chromosome to keep it safe. The phage lacks its own integrase; this task is fulfilled by two host-encoded recombinases (Huber and Waldor 2002). Furthermore, the integrated prophage alone could not export the toxin out of the intact cell. The secretion of the cholera toxin as well as the liberation of the phage for the dissemination of this key virulence factor is mediated by a host-encoded extracellular protein secretion (eps) type II secretion system (Davis et al. 2000). Indeed the key virulence factors TCP and CT are under the control of master regulators, which are encoded in the ancestral Vibrio chromosome. ToxR is regarded as the central virulence regulator and a set of about 60 genes, termed the toxR regulon (involved in colonization, toxin production, and bacterial survival in the host) are coregulated by ToxR in response to external stimuli (Bina et al. 2003).

Adaptation to Human Gut

V. cholerae shows a remarkable adaptation during passage in the human host by changing its transcription profile. In the stool of cholera patients, the bacterium exhibited high expression levels of genes required for nutrient acquisition and motility when compared to V. cholerae grown in the laboratory. Notably V. cholerae excreted by the patients showed a 700-fold higher infectivity than broth-grown bacteria. The bacteria thus adapted their transcription pattern for optimal transmission of the pathogen, thus laying the ground for epidemic spread (Merrell et al. 2002). Oddly, the key virulence genes like TCP and CT were not expressed, while a previously overlooked small gene was highly induced. Is this the program for reentry of V. cholerae into the environment before infecting the next host? Other researchers argued that bacteria recovered from stools are in a different physiological state than bacteria grown in the upper intestine where replication and pathogenesis occur. To clarify this point they reevaluated the transcription profile of V. cholerae in the rabbit ileal loop model (Xu, Dziejman and Mekalanos 2003). Analysis of the transcripts suggests that the in vivo expression pattern represents a response to the stress associated with nutrient limitation, scarcity of iron and oxygen.

The Difficult Relationship of Bacteria with Phages

In the quest for the “human food”, the phage and the bacterium play hand in hand (Figure 7.10). This is not that surprising because the success of the bacterial lysogen is also the success of the prophage as seen from the selfish DNA model (Canchaya et al. 2003b). The phage gets a free lift for its genome into niches of the biosphere, which it would not reach on its own. The bacterium can explore the sequence space carried by a mobile DNA element for genes that increase its survival capacity. Both gene systems cooperate to the detriment of the human host or any host for which the lysogenic bacterium is pathogenic. As both phage and bacterial DNA share the same chromosome during lysogeny, they also have at least temporarily the same destiny. As the prophage can again resume a new replication cycle, which can kill the host, the bacterium plays with
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Figure 7.10. *Streptococcus thermophilus* phage Sfi21 resembles morphologically the famous temperate *E. coli* phage lambda (a). It adsorbs to the cell wall of this yogurt-fermenting strain and injects its DNA into the bacterial prey (b), intracellular phage replication then leads to the lysis of the cell and release of progeny phage, which can then infect new cells. This amplification of phage can quickly kill the bacteria in the fermentation vat, leading to the loss of the product. Phage contamination is a major problem in many industrial food fermentation processes. Sometimes, the phage opts for a different replication strategy by integrating its DNA into the bacterial chromosome—it becomes then a parasite at a molecular level or a selfish DNA.

Fire and the cooperation aspects are still complicated by a parallel arms race between the virus and the host bacterium. To allude to the title of the section, the *V. cholerae* phage has a clear Janus face for the bacterium, but only a grim face for us as the host of the pathogenic bacterium.

Cholera Cycles

Is this really so? Two recent reports by two old hands in cholera research added a surprising twist to the natural history of cholera, which might be Janus’ laughing
cholera face for us. Rita Colwell explored the links between the cycles of infectious diseases and global climate change. In this context she has developed cholera as a paradigm of climate impact on human health (Colwell 1996). The analysis of time series of cholera cases in Bangladesh associated cholera dynamics with the frequency of El Nino-Southern Oscillation events describing temperature anomalies in the ocean (Pascual et al. 2000). However, a review of the literature on many infectious diseases found no unequivocal examples of natural changes in disease severity resulting from directional climate warming per se (Harvell et al. 2002). The cyclic nature of cholera in the Ganges delta region is, however, uncontested. Epidemics usually occur twice a year: a major one after the monsoon and a minor one during the spring.

Phages as Drivers of Cholera Cycles?

J. Mekalanos from Harvard University and B. Nair from the diarrhea hospital in Dhaka, Bangladesh, made fascinating observations that could provide an alternative explanation for these cycles. They found that water samples contained either epidemic \( V. cholerae \) or O1- and O139-specific phages, but rarely both. The time curve of their appearance showed a characteristic cycling as if the accumulation of cholera phages in the environmental water ended the epidemic. As lytic cholera phages are efficient predators of their host bacterium, the observation offers even a potential causal relationship. Most interestingly, phages that were closely related to those viruses which attacked the epidemic strains were released from lysogenic \( V. cholerae \) strains found in the environment (Faruque et al. 2005a,b). During the early phase of the epidemic, phage titer in the environment were low. As the epidemic progressed, an increasing number of patients excreted phage with their stools. At the end of the epidemic, up to 100% of the patients’ stools tested positive for phages and titers could reach up to \( 10^8 \) phage particles per ml of watery stool. Phage might thus be responsible for the collapse of the epidemic. We are now back to old concepts. John Snow linked contaminated Thames River water pumped into the city of London to the observed cholera cases in 1855. When clean water was provided, the cases ceased in London. For cost reasons this environmental engineering approach is not practical in developing countries.

Phage Therapy

Therefore the British army and the WHO used in the 1940s—apparently with some success—an idea developed by Felix d’Hérelle at the beginning of the twentieth century. He proposed to use phages as therapeutic and prophylactic agents against bacterial infections. This idea led to phage preparations by American pharmaceutical companies like Elli Lily in the 1930s. The German and the Red Army used phages in World War II against dysentery and wound infections and US labs conducted promising research in that field until the 1940s when the development of antibiotics replaced most scientific efforts in phage therapy. The idea to use phages was nearly forgotten were it not for a few
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labs in the Soviet Union maintaining this tradition to our days. Currently, there is a growing interest to use the hunger of phages for bacteria as the basis for medicine (Brüssow 2005). The hypothesis that the wax and wane of the cholera epidemics is possibly triggered by cyclic activation of phages from lysogenic cholera strains might be of practical application. Notably, a British medical officer in India added in the 1940s cholera phages to wells, apparently with some success against cholera epidemics. This would be a nice illustration of the Janus faces of cholera phages, which bring virulence factors into V. cholerae making it a potent human pathogen, but which also provide the little helpers that man can use in his fight against this disease. Biology is perhaps always Janus-faced, it is not dominated by the “either–or” as the human mind wants reality, but by the ambiguous “as well as” so disliked by human categorization efforts. Evolution does not think in alternative options, it considers all options for an organism. From the viewpoint of a phage the “either–or” makes no sense. What the cholera phages must follow is the optimal amplification of their genomes if they want to stay in place. Under some conditions it will be more appropriate to associate with a bacterium and to contribute virulence factors to further this cohabitation, while under other conditions it will be more profitable to consider all susceptible bacteria as a prey. Perhaps the best strategy for the phage is to get to the crest of the epidemic wave passively as a prophage and then to use this top position to run the maximal havoc under the bacteria by replicating as a lytic phage. What we see as a Janus face is only our view because we get on and from the hook with these two viral replication strategies, while phages further their progress with both.

From Gut to Blood: The Battle for Iron

Iron Thievery: From Enterobactin to Lipocalin

A pathogenic bacterium that wants to grow in the human body must overcome the formidable barrier presented by transferrin, which keeps the free-iron concentration in the blood as low as $10^{-26}$ M. This concentration is too low to sustain bacterial growth. Similarly, many body secretions contain lactoferrin, namely tears, semen, and human milk, hence its name. Like transferrin it binds two ferric ions and prevents the growth of bacteria. Iron-lactoferrin must also have some bacteriocidal effect as bacteria do not grow after exposure to it even when iron is provided that exceeds the iron binding capacity of lactoferrin. Many aspects in biology are the result of an evolutionary arms race. This is also the case for iron. An E. coli pathogen that causes a bacteremia must cope with the low blood iron concentration. The answer is clear-cut: if transferrin has a binding constant for the Fe$^{3+}$ complex of $10^{24}$ at the physiological pH 7, enterobactin counters with the astronomical Fe$^{3+}$ binding constant of $10^{52}$. Checkmate for the host? Biology would be a poor game if the host would now give up. In fact, if we had not designed countermeasures, our evolutionary survival might have been at stake. Actually, the rescue in this iron fight comes not only from our sophisticated adaptive immune system, but from an old trick also used by bacteria: iron
thievery (Flo et al. 2004). After peritoneal infection of mice with E. coli, the synthesis of lipocalin is part of the acute phase response. The protein is especially prominent where it is most needed: in the liver, which filters everything that comes directly from the gut, and in the spleen, which clears bacteria that escaped the screening in the liver. Mice, in which the lipocalin 2 gene was knocked out, succumb quickly to E. coli infection, while wild-type mice survive the same challenge unharmed. The addition of a recombinant lipocalin expressed from a plasmid to the acute phase serum of the knockout mice restored the bacteriostatic effect. This is nearly a fulfillment of Koch’s postulates for a genetic experiment. The key to the mechanism of lipocalin’s action is that it binds enterochelin specifically with a high affinity (10^{-10} \text{M}). It steals the siderophore from E. coli and starves thereby the bacterium for iron. The effect is quite specific despite the fact that Salmonella, Brucella, and Corynebacterium diphtheriae are also inhibited—they all elaborate an enterochelin-like siderophore. Staphylococcus aureus, however, is not affected by lipocalin 2 because it uses a different iron uptake system. Ferrichrome is not bound by lipocalin 2. Ferrichrome, experimentally added to the system, therefore allows the growth of E. coli in the presence of lipocalin 2, both in vitro and in vivo.

Getting Independent from Iron

It now becomes clear why E. coli uses so many different iron uptake systems in parallel. Other bacteria, like lactic acid bacteria, do not require iron in their metabolism; they can thus grow under extreme iron shortage conditions. Interestingly, lactobacilli make up a nonnegligible part of isolates from blood samples sent to the clinical laboratory for diagnostic purpose. As they lack pathogenic potential, their presence in the blood is surprising and might be explained by their transgression from the gut (where they are a prominent commensal) and their iron sufficiency.

An Adaptive Value of Anemia?

The existence of an iron battle line between bacteria and humans became already apparent to English physicians in the nineteenth century when they tried to treat anemia. Anemia is characterized by a diminished erythrocyte count and reduced hemoglobin concentration. It has three principle causes: enhanced blood loss, and increased destruction or decreased synthesis of erythrocytes. Decreased synthesis is frequently due to insufficient availability of iron. That this shortage could be alleviated by oral iron supplementation was already clear to nineteenth-century physicians. However, in few patients they saw something rather odd: first an amelioration of the anemia — a pale anemic woman got rosy cheeks again. However, the physician’s delight was of short duration. The recovery phase was followed by a rapid decline and the patient died of tuberculosis. The medical background for this case report is clear from our previous paragraph. In cases of iron-shortage anemia, mycobacteria are apparently kept in check by
the lack of available body iron. Once supplemented, mycobacteria could resume their growth, which led to the demise of the patient.

Hemolysins

As the pathogen–host interaction is one of the most dynamic aspects of coevolution in biology, bacterial pathogens have developed a number of strategies to actively deal with the problem of iron shortage in the blood. Iron is found in high concentrations in red blood cells, bound to the $O_2$ transporter protein hemoglobin. How to get to these resources? Many pathogenic bacteria solved this problem with the help of a simple device called a hemolysin. The name already indicates how they do the trick: they simply lyse red blood cells, which leads to the release of the iron-containing blood pigment. The task is not that easy: Enterohemorrhagic E. coli (EHEC) dedicate four genes to its synthesis, the *hylCABD* operon. Its genetic footprints testify that it is a recent acquisition of pathogenic *E. coli*. Its GC base content is much lower than the *E. coli* average and it is associated with other virulence genes. It forms part of a pathogenicity island, a piece of mobile, horizontally acquired DNA. HlyA or α-hemolysin belongs to a family of membrane-targeted toxins also found in other pathogens like *B. pertussis*, the cause of the whooping cough. Hemolysin is synthesized as an inactive protoxin and has to mature by the action of HylC. The latter is an acyl transferase, which adds a fatty acid stolen from the fatty acid biosynthetic pathway. HylA has a distinct domain structure: first a hydrophobic pore-forming domain, then at the center two HylC-binding regions FAI and FAII (referring to the two fatty acids), then a repeat region (RTX), and finally a C-terminal secretion signal. The lipidation of HylA is absolutely required for its pore-building properties in the target cell. However, HylA must first get out of the bacterial cell. This export process is achieved by the next two proteins encoded by the *hyl* operon, HylB and HylD, which assemble in the bacterial inner membrane to form a translocase complex. The toxin export requires hydrolysis of ATP mediated by HylB and contact with the translocase complex. TolC from the outer membrane is then recruited by the transfer complex. TolC is a homotrimer forming a hollow tapered cylinder. One part consists of an α-helical tunnel, which crosses the periplasmic space between the inner and the outer membranes, and a β barrel that spans the outer membrane (Koronakis et al. 2000). In concert with the HylB, D translocase complex, TolC mediates the gated exit of HylA out of the cell. The repeat region of HylA binds first $Ca^{2+}$ and then the glycoprotein glycophorin on the membrane of the red blood cell. An oligomerization of HylA and insertion into the erythrocyte membrane follows. This leads to a ring-like structure visible in electron microscopy with a 2.5 nm inner transmembrane pore. The alteration of the membrane permeability causes lysis and death of the erythrocyte, which provides iron to the bacterial cell. Actually, the lytic action is not limited to red blood cells, but extends to immune cells and epithelial cells. The latter is important since the EHEC strain O157 is noninvasive and remains restricted to the gut. Does HylA target the ferritin stores in the mucosal epithelia? Without being of obvious use to the
food pathogen \(E. \text{coli} \) O157, one of the most feared consequences, the hemolytic uremia syndrome, is in fact linked to the lysis of erythrocytes in the circulation.

**Shiga Toxin**

Why do I tell you this facet of the quest for iron at some length? Actually, \(E. \text{coli} \) O157 is a good example to demonstrate the dynamic nature of the battle for iron. This class of pathogenic \(E. \text{coli} \) has appeared in humans only very recently. The first cases were described in 1982. It got its alternative name STEC (Shiga-toxin producing \(E. \text{coli} \)) from another toxin that it elaborates, the Shiga-like toxin. The experimental character of the O157 \(E. \text{coli} \) toxins and their involvement in the quest for iron become even clearer with the Shiga-like toxins. They come in two forms, Stx1 and Stx2. Stx1 differs from the better known Shiga toxin of \(Shigella \text{dysenteriae} \) by a single amino acid replacement. They belong to the AB-type toxins and are composed of an enzymatically active A subunit which is surrounded by a pentameric B subunit ring (Stein et al. 1992; Fraser et al. 1994). To be fully active the A subunit must be cleaved at the disulfide loop connecting the A1 and A2 fragments. The activation is done by the protease elastase from the intestinal mucus (Kokai-Kun et al. 2000). The B subunit mediates the binding reaction to neutral glycosphingolipids, namely Gb3. Interestingly, in the intestinal Caco2 cells, Gb3 expression is induced by butyrate together with the differentiation of villus cell differentiation markers. Butyrate is produced by the normal resident enteric flora at high concentrations in the human colon, the site of STEC infection (Jacewicz et al. 1995). After binding, the Shiga toxin is internalized by receptor-mediated endocytosis at clathrin-coated pits. Translocation occurs from the endoplasmic reticulum to the cytosol where the A subunit functions as a glycohydrolase. It cleaves a specific adenine from the 28 S rRNA and inhibits thereby the binding of aminoacyl-tRNA to the ribosome. At the end, an irreversible block of protein synthesis results. The toxin action on the intestinal epithelial cells of the colon leads to a submucosa congestion and hemorrhage. The stool shows blood and pus, which led to the alternative name EHEC for these pathogens, enterohemorrhagic \(E. \text{coli} \). Now the likely evolutionary sense of the toxin becomes evident. Again, it could be iron stealing by the bacterium from the decaying erythrocytes in the intestine. This suspicion is reinforced when looking into the transcriptional control of the \(stx \) genes. A functional promotor was identified directly upstream of the \(stx1 \) gene. The activity of this promoter is regulated by the environmental iron concentration via a mechanism involving the iron-dependent Fur transcriptional repressor, which is thought to bind to a site near the promoter (Calderwood and Mekalanos 1987). Only when the iron concentration in the bacterial cell is low and iron becomes growth limiting, the expression of the Shiga toxin is induced. We see here that bacteria have added a new gear into the arm’s race for iron acquisition. This is not an isolated case. Another classical AB toxin is diphtheria toxin elaborated by \(C. \text{diphtheriae} \). Its A subunit is an ADP-ribosyltransferase, which covalently modifies the elongation factor-2, thereby inhibiting chain elongation during protein synthesis (Holmes 2000). The expression of the diphtheria toxin is
regulated via the DtxR transcriptional regulator that binds in an iron-dependent way to the operators of many bacterial genes. Notably, Fur and DtxR are the master regulators of large bacterial iron regulons (genes scattered through the genome, but submitted to a joint expression control). You realize that a major theme of bacterial pathogenicity is the quest for iron.

An Emerging Pathogen: *E. coli* O157

I promised you an insight into the experimental character of bacterial evolution. Until now the iron acquisition mechanisms of *E. coli* seem rather cute. However, a deeper look into the system reveals that the system is not yet poised. Actually, STEC has despite its name no export mechanism for its toxin and thus suffers a drawback not known to Hy1A. There are other indications that the Shiga toxin is a newcomer to STEC. stx1 and stx2 are encoded on prophages; they are thus not part of the bacterial genome, but came into bacteria via the integration of the genome from temperate phages. Numerous bacterial pathogens owe at least some virulence factors to such integrated viral genomes (Figure 7.11) (Brüssow et al. 2004). The export mechanism is also provided by the phage genome. Directly downstream of the stx genes, you find the phage lysis cassette consisting of a holin and a lysin. The holin builds a pore into the inner membrane of *E. coli* through which the lysin can exit from the cell. The lysin attacks the cell wall and the weakening or digestion of the cell wall leads to an osmotic explosion of the cell if a major part of the cytoplasmic content had not already passed through the holin pores. With that cell lysis the Shiga toxin is also released. The problem is: the bacterial cell, which produced the toxin, is dead and by any definition a dead cell cannot any longer profit from the iron supplied by the intestinal hemorrhage induced by the Shiga toxin. The situation is not totally unknown to bacteria. As the infecting bacteria are clonally related, the arguments of the selfish gene do not apply to the interaction between these bacteria. If a minority commits suicide, the majority of their genetically identical brethren can profit from the iron rain into the intestine.

Colicins

A comparable situation was already described for colicins. Colicins are proteins encoded on the plasmids of *E. coli* that are exported and kill closely related, but not identical bacteria. The idea is a chemical club that is used to wipe out related bacteria that are by definition the most potent competitors for the nutritional resources in their ecological niche. Identical bacteria also carry on their plasmid an immunity function that protects the cell against the toxic effect of the colicin (colicin E1 permealizes the cytoplasmic membrane, E2 and E3 degrade DNA and rRNA, respectively). The selfish gene argument is thus satisfied under this condition. The medically widely applied antibiotics are also part of these chemical clubs used by microbes to clear their environment from nutritional competitors. Bacteria were thus under the selective
force of antibiotics long before the compounds were used in medicine. The corresponding immunity factors, the antibiotic resistance genes, were also already at hand, which explains why some antibiotics have only a short lifetime after introduction in medical practice. In the case of colicins, only some bacteria produce them and die for the benefit of their congeners. STEC might have accepted death of some cells by prophage induction to the iron benefit of the remainder.

**Recruiting Bystander Cells**

*STEC cells have “discovered” another way around the iron problem and the suicidal toxin production. As the toxins are encoded on a phage, another solution*
is at hand. A few STEC induce the prophage, which leads to the production of infectious phages from the lysed cell. This phage can then infect bystanders like the commensal E. coli in the colon of the infected host. These cells then undergo lytic infections, which lead to an amplification of Shiga toxin production without harming the STEC cells. They are actually “immune” to the action of the released phage via the immunity repressor, which is constitutively expressed from the resident prophage in STEC. While still somewhat speculative, this model of STEC infection is backed by a number of recent biological observations (Gamage et al. 2003) and can explain a number of otherwise paradoxical clinical observations (like the detrimental effect of antibiotics in STEC patients).

Pathogenicity as Nonadaptation to a New Host?

In fact, STEC might be a good example for a newly evolved pathogen. Pathogens making a hard ride with their host are frequently considered a newcomer in that system. The systemic effects of a noninvasive STEC like the kidney failure leading to dialysis in pediatric patients might be an evolutionary unwanted unbalanced side effect of an intestinal pathogen. This conclusion is not only backed by the recent emergence of EHEC in humans. The molecular archaeology of O157 also suggested the sequential acquisition of the rfb genes (encoding the O-serotype determinants), phage stx2 and then phage stx1 within perhaps 100 years. In accordance with the theory, O157 E. coli strains are not pathogenic in the intestine of cattle—their natural host and reservoir—and they have arrived in humans only quite recently, probably mediated by some changes in food consumption or processing.

Viruses Going for Gut or Genome

**Portrait of a Killer Virus**

Perceived and Real Killers

Many viruses feed on us, we call this phenomenon infectious disease, but from the viewpoint of viruses we are simply the substrate for their growth, providing nutrients and a good deal of biosynthetic activity. In this section I deal with rotavirus. You might raise your eyebrows as you have not yet heard from this killer, but this is a question of media attention. There is a strong bias between perceived threats and real killers (Glass 2004). We are frightened by West Nile virus, Ebola, and SARS headlines in the newspapers because they remind us that we are not really on the top of the food pyramid threatened only by our own suicidal instincts. These viruses remind us that we are perhaps not directly food, but at least a suitable substrate for our viral predators. Ebola—how dreadful the disease might be—isn’t an efficient killer. The real killers are other viruses: influenza that kills each year 37,000 persons in the United States and had and still has the potential of great pandemics as demonstrated by
the 1918 Spanish Flu, which killed more people than World War I. Rotavirus (RV), although less well known, kills yearly about 500,000 children and this on a regular basis. As these deaths occur mainly in the developing world and go along with a disease that we are used to see as a nuisance and not a real threat, namely diarrhea, its death toll remains unnoticed. However, children in the industrialized world also become growth substrate for RV. As they are quickly hospitalized and generally well nourished, the death rate is very low.

Rotavirus Epidemiology

To put this quest for food by a single virus in perspective: every child, irrespective of its place of birth, experiences during its first years of life a few episodes of RV infections, the first episode tends to be symptomatic, it causes a diarrhea frequently associated with vomiting. In this respect, RVs are part of what epidemiologists call—somewhat floppily—a democratic infection. It is very hard to find people that lack serological evidence of RV exposure: you have to go to a tribe living in a remote area of Amazonia or you must follow a missionary on isolated islands of Oceania, who apparently seeded an RV epidemic on all islands he visited. Approximately 1 in 10 children in the industrialized world sees a physician for this diarrhea; 1 in 100 gets hospitalized. In fact, during the RV winter season, about 5% of all pediatric hospitalizations are due to RV diarrhea. On a purely quantitative basis, we are the feeding basis for a massive RV replication. The situation is worse in the developing countries where RV meets children already weakened by malnutrition. In many parts of the poorer corners of the world children experience from 5 to 10 episodes of diarrhea per year. Thus a substantial part of the world children population spends up to 15% of their time on diarrhea. This condition acerbates malnutrition, and a negative spiral of growth retardation sets in. Of course, not all of these infections are RV-induced. Many bacteria and parasites actually target our gut as a feeding substrate. However, RV infections in children from developing countries tend to be clinically more severe than non-RV diarrhea. There is not much consolation in the fact that we are not the only target of RV, we share this destiny with the young of many mammals and birds.

Transmission

Let’s look a bit into the feeding strategy of RV. Gastroenteritis agents generally have a fecal–oral infection route. The diarrhea agent is explosively discharged with the stool and evolutionary biologists interpret this as an adaptation to efficient dispersal. The fecal microbe then contaminates water or food sources and is thus recycled via the oral infection way back into the intestine of another subject where the next round of replication resumes, thus maintaining the agent in the population. As the drinking water in the industrialized world is chlorinated and the food for children is frequently produced industrially, it is not evident why RV infections are so prevalent in the northern hemisphere. Here epidemiologists tell us that RV behaves like a respiratory infection; in the United States, they
even appear to be blown with the winds if one looks to the kinetics of the annual RV epidemics starting on the west coast in late fall, crossing the continent, and running out on the East coast in late spring. This peculiar pattern was interpreted by the fact that small doses of RV might be sufficient to get infected, while about $10^4$ vibros are needed to contract cholera.

**Histopathology**

Once in the intestine, RV infects the mature enterocytes covering the villi of the intestinal mucosa. RV-induced diarrhea occurs early after the infection before significant histopathology is evident. In experimentally infected piglets a patchy viral antigen distribution is seen in the enterocytes with a decreasing intensity from the duodenum over the jejunum to the ileum. At 12 hours post infection, an early profuse secretory diarrhea is the probable consequence of the production of a viral enterotoxin, the nonstructural protein NSP4 (Ball et al.1996). The elaboration of a toxin is an absolute rarity in viral infections. The pathological effects of viral infections are normally the consequence of the cytopathic effects of the viral multiplication that leads to the death of the infected cell. In rare cases viral pathology is also the consequence of a derailed immune reaction against the invader. The cytopathic effect of RV infection becomes clear at 24 to 72 hours post infection. The viral antigen is widespread in the mucosa and viral titers are high in the intestinal lumen. The villi become atrophied and blunted. Signs of inflammation are seen in the mucosa, the epithelial cells are vacuolated, and the lamina propria is infiltrated by mononuclear cells.

**Pathophysiology**

The loss of the differentiated enterocytes leads to maladsorption. The lactase activity is lost and the impeded absorption of lactose leads to a further osmotic drag into the intestinal lumen, which aggravates the secretory diarrhea. In that phase, children show various degrees of dehydration ranging from sunken eyes and fontanella to loss of turgor in the skin. If uncorrected, the water and electrolyte loss can lead to acidosis, neurological disturbances, and death. It is absolutely critical that the children are at that moment rehydrated. The World Health Organization advocates an oral rehydration solution consisting of glucose and sodium chloride. The glucose is a nutrient, but its main function in the solution is to lead to a concomitant import of sodium into the intestinal mucosa. The trick is actually that glucose transport from the gut is, in the normal physiological situation, powered against the higher glucose concentration within the enterocytes by the cotransport of sodium downhill the concentration gradient (sodium is higher in the lumen than in the enterocyte). Other sodium transporters are frequently nonfunctional in diarrhea patients; sodium without glucose would thus not be absorbed. In severe cases the glucose–sodium solution must be instilled intravenously. With these measures death can be prevented and the patient recovers within days.
Self-Limited Infection

The acute infection and diarrheal disease normally resolves within 7 days and the intestinal mucosa again looks normal. This *restitutio ad integrum* is explained by the great regeneration power of the intestinal mucosa. Immature enterocytes are constantly born in the crypts of the mucosa and the differentiating enterocytes travel from the crypt to the top of the villi from which they slough off in a physiological growth cycle. In this way, the entire intestinal mucosa is renewed in every human being within days. Were it not for the water and electrolyte disturbances, RV infections would only be a rather benign gut disease. Not all viral infections of the gut are that respectful. Some enteric coronaviruses (the family to which SARS belongs) target the regenerative crypt cells for viral replication. When these cells die as a consequence from viral infection, no new enterocytes are regenerated and the intestinal villi atrophy permanently. The absorption is permanently interrupted and death is the logical consequence. Fortunately, this is not a human disease, but occurs only in swine (transmissible gastroenteritis virus, TGE).

Target Specificity

Many viruses are very selective for their target cell. RV exclusively uses the enterocytes as support for its propagation. Only in experimental animals with combined immunodeficiency, liver infections by RV were reported. How does RV target suitable cells? The virus is not only delicately poised towards its target cell, the enterocyte, it also targets apparently a developmental regulated viral receptor, as RV infection is a disease of the young animal and RV is, under natural conditions, very species specific. Only few human individuals were infected by bovine RV in the field despite the fact that humans can be experimentally infected with bovine RV (this was actually the first-generation RV vaccine; Roberts 2004). Only a single case of an avian RV possibly infecting a calf was reported (Brüssow et al.1992).

Rotavirus Genome

To get an answer, we must look into the molecular organization of RV. Its genome appears like a messenger from another world. Actually, its total genome size consists of about 19,000 bp divided into 11 segments of double-stranded RNA ranging from 3,300 to 660 bp. Each segment codes for a single protein except segment 11, which codes for two nonstructural proteins via frameshifting. There are noncoding regions at both ends of these simple one-gene chromosomes that have to fulfill a number of tasks. They contain a minimal promoter and transcriptional enhancers, signals for replication and translational enhancers.
Figure 7.12. Double-layered rotavirus particles containing the double-stranded RNA genome (bottom) and triple-layered rotavirus particles lacking the RNA genome ("empty capsids") (top) as seen by negative stain electron microscopy. Both particle types are noninfectious, the first because it lacks the viral antireceptor, the second because it lacks the genetic information.
Translation

The mRNAs transcribed from them are capped, but not polyadenylated and need the help of the viral NSP3 (non structural protein 3) for translation. NSP3 associates with the poly(A)-binding protein of the translation initiation complex of the ribosome, which leads to the replacement of the cellular mRNA by RV mRNA. In this way, shortly after infection of the cell, the ribosomes synthesize RV proteins nearly exclusively. *The pirate has taken control of the boarded ship.*

RNA Replication

The viral proteins NSP2, 5, and 6 are associated with the so-called viroplasms, large virus core–building factories with nearly a crystal structure where RNA replication in the nascent virion cores takes place. NSP2 has been investigated in some detail (Jayaram et al. 2002). NSP2 oligomerizes into octamers, two stacked rings of four proteins leaving a central pore of 35 Å, not unlike a doughnut. The authors suggest that this NSP2 complex functions as a molecular motor during genome replication and packaging. Viral RNA binds to the external groove, the viral polymerase in the central pore.

The Enzymatically Active Viral Particle

Different virus-like particles could be reconstituted with recombinant RV proteins. Particles assembled from VP2 resembled viral cores and those assembled from VP1, 2, 3, and 6 resembled double-layered empty viral particles also observed in infected cells. The core of two related viruses, namely reovirus (Reinisch et al. 2000) and bluetongue virus (Grimes et al. 1998), was solved by X-ray analysis and the double-layered RV particle was analyzed by cryo-electronmicroscopy, and radial-density profiles were computed. This allowed a location of the different proteins in the RV particle (Prasad et al. 1996). The center is built by a dense convolute of RNA strands. In the better-resolved bluetongue virus, the RNA helices are tightly coiled, reminiscent of the dense DNA packaging observed in the head of phage particles. Only two empty volumes were observed—the likely place of the transcription complexes in bluetongue virus. A thin shell of VP2 is observed surrounding the central RNA convolute. It is overlaid by a more robust VP6 shell and underlaid at specific symmetry positions by a flower-like structure consisting of VP1, the RNA polymerase, and VP3, the mRNA capping enzyme. The VP2 and VP6 shells leave a hole over the flower cups through which the mRNA is extruded (Reinisch et al. 2000). *Why all this complicated structure and the associated enzymes? The answer is simple: the invader has to first make cautious steps that the cell does not detect the presence of a pirate on the ship. The regular crew of the cellular ship has a simple cue: they screen the ship for double-stranded RNA and when they detect this sign of an unfriendly takeover a vigorous defense response is set in motion. RV and its relatives therefore avoid presenting free dsRNA at any step of their multiplication cycle. dsRNA is always hidden in a protein shell.*
Transcription takes place from the uncoated viral particle directly after entry into the cell. Uncoating means, however, that only the outer protein shell consisting of VP4 and VP7 is blown away, induced by the low Ca\(^{2+}\) concentration in the cytoplasm. The double-layered particle thus starts with transcription and the viral mRNA is capped in exactly the same way as cellular mRNA. The cell has no cue to detect it as foreign RNA. In later steps, the mRNA also serves as a template to synthesize the complementary strand. Before replication starts, the mRNA is complexed with proteins in the viroplasm—again no dsRNA becomes visible to the cell. In this way the cell is overrun before it actually takes notice of the unfriendly takeover.

The Infectious Viral Particle

Now RV needs the final brush-up, the outer layer with the proteins that allow the targeting of the next prey when the newly made RVs are released from the dying cell. This process takes an interesting detour. NSP4, which we mentioned already as the first viral enterotoxin, has still another job: it is a glycoprotein that inserts into the membrane of the endoplasmic reticulum, attracts the nascent double-layered particle, and leads to the transient build-up of an intracellular membrane-enveloped particle before the outer shell proteins VP4 and VP7 take their final position in the infectious triple-layered particle. VP7 assembles as a third shell surrounding the mature virion. This layer is interrupted by spikes, built by VP4 that project beyond the VP7 shell. As VP7 and VP4 are the most exposed proteins they become the target of the immune system leading to neutralizing antibodies and cytotoxic T lymphocytes. As they are under immune selection, it is not surprising that RV differentiated in more than a dozen G serotypes (VP7 antigens) and an equal number of P serotypes (VP4 antigens).

Viral Spikes and Cell Entry

The X-ray structure and the structural rearrangements of VP4 relevant for the membrane penetration have been recently resolved (Dormitzer et al. 2004). Three distinct conformations correspond to steps of RV entry into a new target cell. When released from the previous cell, VP4 is noncleaved. In the intestine, trypsin provides the first cue: this pancreatic enzyme signals the appropriate ecological niche and triggers a first structural change by the tryptic cleavage of VP4. It is critical for any virus to make sure that it is really on the target. If the penetration process is initiated on a nonpermissive cell, the infectivity is lost. RV apparently uses a cautious strategy: first it searches with the viral spike protein the primary receptors, which are sialic acids in the context of gangliosides of the cellular membrane (Delorme et al. 2001). The following steps are only known in their outline: when the appropriate ganglioside is bound, the spike ejects the cleaved tip only held by noncovalent bonds. This process unmasks a hydrophobic region in the spike that inserts into the host membrane. The viral spike protein then interacts with the secondary receptors, a specific integrin. Then RV rolls apparently to still another tertiary receptor consisting of a complex of three
membrane proteins. *This multistep entry of RV into the cell has been likened to the steps of a minuet dance of court balls where a dancer moves in small steps along a line of contradancers sharing frequent kisses* (Lopez and Arias 2004). After these confirmations of the right target cell, the spike protein folds back and creates a breach through which the RV enters the next cell. Notably, this entry process of a nonenveloped virus closely resembles that determined for enveloped viruses (Modis et al. 2004; Gibbons et al. 2004) unifying principles are thus also found in the biological world of viruses.

**Bluetongue Variation**

An interesting variation of this entry scheme is provided by the bluetongue virus, a cousin of the RV. It causes a hemorrhagic fever in sheep characterized by prolonged viremia, which is ecologically necessary as the disease has to be transmitted by blood-sucking insects. To avoid the onslaught of the immune system in the blood, the bluetongue virus (the name describes a characteristic clinical feature in sheep) hides out in erythrocytes. As it has to replicate in both an invertebrate and a mammalian host, it is confronted with the problem of how to build two recognition systems. Bluetongue virus has solved this problem elegantly: the triple-layered particle contains, as in the corresponding RV particle, the recognition proteins for the mammalian cell receptor of this virus, while the double-layered particle from bluetongue virus (which is noninfectious in RV) is infectious for the insect cell.

**A Remarkable Design . . .**

*We have now seen what quest for food means for the RV. RV is for the standard of RNA viruses already a complex virus. RNA viruses have to solve a few biochemical problems with the constraint that RNA probably does not allow the use of really large genomes. One additional problem is the need to contribute the enzymes for their replication as such enzymes are unknown to the cell. The virus has to think on its camouflage and then an efficient takeover of the cells in order to build a stable transport vehicle for its own genome. Finally, they need a carefully poised protein apparatus to find and then breach into the next susceptible cell. I wonder whether molecular biologists would not despair if they are asked to achieve all this with a mere 18,500 bp of genetic information when starting from scratch. The common argument is that of R. Dawkins “Blind Watchmaker,” evolution had very long time periods to tinker around to achieve the solution that we see now. How long is actually a contentious issue in virology. Animal virologists are used to phylogenetic trees, but no fossil record or molecular clock exists for viruses.*

. . . **Coming from Where?**

*How old are dsRNA viruses? Are they living fossils from the early genomes of the RNA world? Is RNA double-strandedness a solution to confer some error*
reduction in information storage? Except for the proteins involved in the camouflage business, no obvious links exist to cellular proteins, making it not very probable that RV evolved from cellular genes. The exotic chemical structure of their genes makes this hypothesis from the start unlikely. On the other hand, RV has an extended family tree. RVs are known from mammals, birds, and fish. The family Reoviridae is found not only in vertebrates, but also in insects (orbiviruses, coltiviruses) and plants (phytoreoviruses). More distant relatives are even found in prokaryotes as demonstrated by the Pseudomonas phage $\phi$ 6 (Karrasch et al. 1995). However, the phage differs in important details from RV. It contains an internal lipid membrane (but recall the NSP4-associated transitory membrane of RV) and only three dsRNA segments that each encodes 4–5 proteins.

A Glimpse into the World of Retroelements

HIV: A Newly Emerged Old Virus

Evolutionary biologists state that old host–parasite relationships are characterized by a rather benign nutritional interaction. The parasite is best served by a surviving host because it continues to provide a good growth substrate. If you meet deadly human microbes, you might expect to see newcomers into the host–parasite relationship. Such a great killer of humans is HIV. At first glance HIV is not a likely reference when you want to explore old relationships reaching back into the evolutionary past. The earliest isolate of HIV dates from 1959 in Kinshasa. This sounds terribly recent and fits with two other observations. The AIDS epidemic is so dramatic that it could not have been overlooked in the past. AIDS is thus most likely a new disease in the medical record. Another element fitting with the recent origin of HIV is its mutation rate, which has been estimated to $10^{-2}$ substitutions per site per year. This fast rate is the product of the high mutation rate of its reverse transcriptase and the rapid replication rate in human victims (Sharp 2002).

The Ritual of Reverse Transcription and Provirus Integration

However, other characteristics point to an ancient origin of HIV. Its genome is RNA, actually two identical (as far as HIV enzymes can achieve high fidelity) strands of single-stranded RNA, which are linked by noncovalent bonds. Furthermore, virions contain tRNA$_{Lys}$ bound to the genome RNA, where it serves as a primer. With the help of its pol gene the viral genome shuttles between RNA and DNA forms. Integration of the DNA provirus into the human chromosome is an obligatory step in the replication cycle of HIV. The course of reverse transcription is complex and involves a lot of molecular acrobatics. A single-stranded viral RNA genome is copied into a double-stranded viral DNA genome. The viral integrase then carries the linear DNA copy into the nucleus, nibbles away two nucleotides from both ends, creating 3’-recessed ends. Then
follows a staggered cut in the chromosomal DNA, strand transfer, and repair of the cuts. This is a complicated molecular dance, which is with small variants faithfully executed by the various retroviruses. *This frozen ritual has the smack of a genetic element that stems from the transition period of the RNA into the DNA world.*

A “Complex” Retrovirus…

Like other retroviruses HIV shows the usual gene constellation *LTR–gag–pol–env–LTR* (large terminal repeat-group specific antigens–polymerase–envelope). Retroviruses with this genome organization are widely distributed in vertebrates. HIV-1 and the other infectious human retroviruses belong to the “complex” retroviruses, which are characterized by a set of six regulatory genes surrounding the *env* gene. These extra genes were new to retrovirologists and they made life very hard to scientists and prevented up to now the development of an efficient vaccine despite enormous research efforts. *To put this dilemma in perspective: the HIV-1 genome is only about 9,400-nt long, while far more than 10,000 research papers have been written on this virus since its discovery as the cause of AIDS in 1984. More than one paper per nucleotide or if you look at AIDS conferences, more than one researcher per nucleotide. It is astonishing that despite this human intellectual investment, the virus still slips through our hands. The grim perspective is that in many parts of the world the epidemic will run full course and might only come to a standstill when it has selected a genetically resistant or—in the more optimistic version—educated human population.*

… Targeting Our Defense System

AIDS has another lesson for us. HIV targets for its replication the CD4+ T-helper/inducer subset of the lymphocytes as its food or growth substrate. As these cells play a crucial role in the immune defense of the body against microbial invaders, the victim loses the most important line of defense and becomes helpless to fight HIV. Notably, AIDS patients suffer and finally die from attacks by other microbes if not supported by substantial and costly medical interventions. Strikingly, many of the coinfecting microbes were pretty unknown to clinical microbiologists before the AIDS epidemic. This observation shows that a major duty of our immune system is to fence off microorganisms that want to prey on us. Microbial predators of all kinds surround us, but we do not realize it because of our immunological armor.

Ancient Proviruses

Killing a host population is only one option for a rogue virus. Other retroviruses opted for an even more hideous strategy: they succeed in infecting germ cells and in integrating their provirus stably into the host chromosome. This integration can lead to the vertical transmission of the provirus via germ cells. Sequences that resemble endogenous retroviruses represent about 0.1% of the
human genomic DNA. These are quite sizable numbers. HERV-H for human endogenous retrovirus integrated into the tRNA histidine gene comes in about 1,000 copies per genome, followed by HERV-K and HERV-E with 100 copies in the tRNA_{Lys} and tRNA_{Glu} genes, respectively. They represent “fossil” infections and insertion into the germ line that occurred in the evolutionary past. None of them belongs to the “complex” retroviruses, possibly underlining the more recent origin of the latter. There might be a subtle interplay between exogenous and endogenous lifestyles. As long as a “new” exogenous retrovirus meets a virgin susceptible population, it has an essentially unlimited supply of target host individuals. Its spread will be horizontal transmission of an exogenous infectious retrovirus. After a while viral interference will develop, the target cell will already carry a provirus from a related family, which will prevent the superinfection. The possibilities of horizontal spread become limiting and the retrovirus will be forced into vertical spread through genome colonization in germ cells. The hit-and-run lifestyle of the exogenous virus is replaced by an intimate, long-term association between the provirus and the host.

Evolutionary Trade-offs

Provirus genes that have a negative effect on the host or are neutral with respect to selection will be lost. This hypothesis actually neatly explains what happens with endogenous proviruses: ancient proviruses tend to be defective, and accumulate stop codons and deletions. If a retrovirus ORF has a beneficial function, it might be maintained. Syncytin is involved in human placenta morphogenesis. It represents a captured *env* gene from an endogenous retrovirus (Mi et al. 2000). An HERV-E insertion provides an LTR promoter for pleiotrophin expression in the placenta (Schulte et al. 1996). Another HERV-E is inserted upstream of a pancreatic amylase gene and provides a salivary gland specific enhancer that allows amylase secretion already in the mouth, thus conferring a sweet taste to a cereal-rich diet for humans (Ting et al. 1992).

Provirus–Prophage Analogy?

*These observations with proviruses show striking parallels with the situation of prophages in bacteria. However, beyond the fact that retroviruses and temperate phages can integrate their genomes into the host chromosome and leave its replication to the diligence of the cell, no other obvious links exist between both viral systems. For example, the prophage and provirus integrase do not share sequence similarities and their genome organization is totally unrelated.*

Gypsy

What is the evolutionary reach of retroviruses beyond vertebrates? Retroviruses with the usual gene constellation *LTR–gag–pol–env–LTR* were also found in *Drosophila*, where they were described as Gypsy element. Gypsy is transmitted
via the germ line as a provirus and is under the control of the host gene \textit{flamenco}. Putative endogenous retroviruses are common in insects, but Gypsy in \textit{Drosophila} is the only invertebrate retroelement for which infectivity has been shown (Kim et al. 1994). With gypsy we got on a slippery road. Retroviruses are only the tip of an iceberg. Gypsy is also classified as an LTR retrotransposon. The LINE or Alu and SINE sequences making up so much of the human genome sequence (see below) are classified as non-LTR retrotransposons and retrotranscripts, respectively. Even bacteria contain retroelements, where they are called retrons. The list is by no means exhaustive (for a review see Bushman, Lateral DNA Transfer: Mechanisms and Consequences, Cold Spring Harbor Laboratory Press, New York, 2002). The function of many of these elements is unclear. Some might in their current state only represent purely parasitic DNA.

\textbf{The Sense of Life}

We touch here an eminent philosophical question of modern biology that also matters in a quest for food survey. Classical philosophers and founders of religion have come up with theories about the goal of life. In our robust anthropocentric (a biologist would say egocentric) view, most hypotheses deal with the goal of human life. Many theories link these goals to ethical commitments or our relationship with God or the transcendent. As natural scientists, biologists should not have a strong opinion on the transcendental, whatever their private opinion on the subject. At an individual level any opinion is allowed, even necessary that allows you to find your personal place in the cosmos during your life time. You can not wait that future centuries of scientific research or philosophical thinking do provide you an answer. Two conditions for a fruitful discussion between science and religious belief are necessary. As a natural scientist you should accept that the transcendent is outside of the framework of your science. Natural scientists start with the working hypothesis that Nature follows only physico-chemical laws. The relative explicatory success of this approach as demonstrated by the current scientific research is, however, by no means a philosophically valid proof that the transcendent does not exist. Scientists should – already from a methodological viewpoint - refrain from strong opinions on religious beliefs. Religious people should, on the other hand, likewise refrain from meddling with the philosophical basis of natural sciences as a working hypothesis to explain the natural world around us. Religions are not a method for an alternative explanation for the natural world around us. It is not the role of Christian faith to dispute the evidence for fossils, geology and the evolution of species. True religious beliefs should provide us with indications how the world should be organized in face of the transcendent, which reaches into our life as a personal or collective experience. Religions should not be against reason, but beyond reason. Where human reason can meaningfully reach, we should let it run its course. Where science cannot any longer count, observe and measure and where we need an answer in our life time, religions might provide us (including the scientist) an answer. The founder of the Christian faith saw these two faces
of the world when telling people to give God what belongs to God and to give Cesar what belongs to Cesar. High profile scientists and theologians should meet to define the borderline between science and religious beliefs (see H. Küng Der Anfang aller Dinge-Naturwissenschaft und Religion, Piper 2005).

Back to biology and reasoning concerning the sense of life within the limits of its framework. Ever since Darwin, biologists they raised their voices that human life is but a small contribution to life. Life must have a “sense” in the absence of ethical and religious terms. The latter terms depend on the presence of a self-conscious intelligent mind or—to use the formulation of the Bible—a mind which knows to distinguish the good and the evil. In the history of life, such minds are a very recent development, which hardly looks back more than 100,000 years. To formulate it agnostically without hurting religious feelings: what was the sense of life left in the geological record and represented by the current life forms before the arrival of human consciousness? Biologists came up with some answers. To come to grip with this complex question, let’s concentrate on some key arguments in this debate.

From Thomas to Darwin

Thomas of Aquino was not really a biologist, but this great Christian thinker of the thirteenth century had a sound interest in the natural world surrounding him. He counted the instinct to preserve the human race as one of the natural inclinations of our species. The preservation of human life became one of the prime goals of human laws and the task of the society was to guarantee this right of physical integrity for the individual. Species conservation was a main goal and this gave us the right to eat the sheep. Species were perceived as immutable until Darwin. He finally perceived that species developed, split into new species, which competed for a place under the sun. The picture got very dynamic and the extant species were seen as the descendent of a long series of extinct species. Although Darwin knew about the power of breeding for the species issue, he had no notion of genetics because the Austrian monk Gregor Mendel missed to send him a letter describing the experiments he did with peas in the garden of his monastery. Due to this handicap, Darwin’s reasoning on the physical basis of heredity had to remain vague.

The Impact of Genetics . . .

The marriage of evolution and genetics had to wait and took place only in the 1930s when the geneticist R.A. Fisher prepared in his book The Genetical Theory of Natural Selection a synthesis of the Darwinian thinking on evolution with population genetics. Now the individual took the place of the species as the actor in evolution. The next step was taken in the 1960s by W.D. Hamilton and G.C. Williams, who emphasized the role of the gene in evolution. This development is only a logical consequence of the population genetics set out in the 1930s. The gene took center stage in the writings of J. Maynard Smith and R. Dawkins in the 1970s.
The discussion in biology has thus definitively taken a reductionist slope from life → species → individual → gene. Small wonder then that molecular biologists like L. Orgel and F. Crick still went one step further to the basis of modern biology when they formulated the selfish DNA hypothesis in the early 1980s (Orgel and Crick 1980; Orgel et al. 1980). This hypothesis rests on the observation that in higher animals and plants, genes are sparsely distributed on the chromosomes while in prokaryotic genomes between 85 and 95% of the DNA is actually encoding proteins. This percentage is only 1.5% in the human genome. Many biologists looked at the noncoding repetitive DNA as “junk.”

From Junk DNA . . .

This interpretation is not farfetched: much of this DNA is indeed derived from mobile elements, 45% of the human DNA would fall into the category of mobile DNA or their action. LINE elements comprise about 20% of the human genome. LINE is an acronym for Long INterspersed DNA sequence Element. They are typically several kilobases long and are found in thousands of copies per genome. Their generic structure is quite revealing: a 5’ untranslated region (UTR) is followed by an orf that encodes Gag-like functions. In some insect elements this Gag still detectably resembles nucleocapsid proteins from retroviruses. Then follows a second orf combining an endonuclease, a reverse transcriptase, and an RNaseH function. However, no sequence similarity with retrovirus RT is observed. RT enzymes are one of the most divergent sets of enzymes known, suggesting that RT is among the evolutionary oldest enzymes. This interpretation is not incompatible with its role it probably had to play in catalyzing the transition from the RNA into the DNA world. The LINE ends with a 3’ UTR and a polyA tail. Many LINE are truncated and rearranged. Another repetitive DNA is called SINE, which stands for Short INterspersed DNA sequence Element. They range in length from 130 to 300 bp and occur as isolated dispersed DNA elements throughout the genome. The most frequent representative of SINE is the Alu sequence, so called because it contains a single AluI restriction site. This 300-bp sequence shows sequence relatedness to the 7SL RNA. Alu sequences occur in more than a million copies in the human genome and thus add another 11% of “junk” DNA to the human genome. Proviruses, DNA transposons, and pseudogenes, derived from the action of the retrotransposon machinery on cellular mRNA, add to the rest of the mobile DNA in the human genome. Of course, all this “junk” DNA reflects our current insight into our genome organization. We have seen that nature is an opportunist, even the “junk” DNA might have taken some function in our genome. For example, in many eukaryotes the centromeres of the chromosomes contain abundant mobile DNA elements, which may have found a useful role as architectural elements. In fruit flies, telomeres of the chromosomes are formed by LINE-like DNA.
... to Selfish DNA

However, the mainstream interpretation is still that of Orgel and Crick, where selfish DNA seeks to achieve Darwinian success by expanding its copy number, while it does not contribute a selective advantage for its host. They profit from the permissiveness of higher eukaryotes with respect to the genome size. No such selfish DNA could develop in prokaryotes, which strive to an economy in their genome size, which rarely goes beyond 10 Mb.

An informational molecule wants world power? What is the take-home message from these considerations? First, it confirms that there is a grain of truth in the word of some researchers who declared that “humans are descended from viruses as well as from apes.” Second, I think this seems to mean that the quest for food is not a hot pursuit in its own sake. Having a metabolism and eating is not a biological goal in itself, it is only a means to another goal. And this ultimate goal is to remain on the scene as an informational macromolecule, where the nucleic acid sequence spells out your identity. J. Monod said once that the ultimate aim of a bacterium is to become two. One could paraphrase that the ultimate aim of life is defined by the zeal of a nucleic acid molecule containing a specific sequence to fill the biosphere. For a philosopher this might seem a minimalist goal, but for many biologists this hypothesis has a strong explicatory power. In his book The Selfish Gene R. Dawkins showed in an eloquent way how far this basic idea reaches out from the inner realm of biology into the understanding of human behavior.

There is no inherent unidirectional trend in evolution to develop ever more complicated life forms with ever more sophisticated eating strategies. It is obvious that our world is biologically more complex than the Archaean Sea or what it was during the Cambrian Revolution. However, we are still surrounded with reductionist organisms. They achieve their goals with minimal means. Cheaters in the quest for food come in many forms. The LINEs and SINEs in our chromosomes that get replicated at our cost without spending time in the quest for food are only one extreme, but at the same time the most successful form.