Chapter 13

The Crowded Cytosol

“If you can talk with crowds and keep your virtue,
Or walk with Kings – nor lose the common touch,
Yours is the Earth and everything that’s in it.”

Rudyard Kipling [1]

While, given the expansion of the world population, it cannot be guaranteed that it will always be so, currently one accepts as a temporary discomfort short periods of crowding, as in a crowded elevator or subway train. However, if, like most intracellular macromolecules, one were both blind and deaf, the need to communicate by touch might make crowding an option of choice. It seems likely that the first cells to evolve soon discovered the advantage of intracellular crowding, which persists to this day [2]. Indeed, the physiological environment of enzymes is very different from the environment we can normally create in the test-tube.

The French microbiologist Antoine Bechamp reported in 1864 that enzymes (“ferments”) in yeast would work outside of the cell of origin [3]. More than anything else, this demystified the cellular “protoplasm” suggesting that, in principle, it should be possible to take a cell apart and then reassemble it from its individual components, as if it were a clock. So enzymes were purified and characterized. It even became possible to purchase enzymes “off the shelf” as protein powders. The study of their abilities to convert target molecules (substrates) to other molecules (products) kept biochemists busy for much of the twentieth century, and the fundamental chemistry of life emerged.

For this, the enzymes had to be dissolved in suitable salt solutions. But it is difficult to dissolve proteins at concentrations higher than 10 mg/mL. In contrast cytosolic proteins are collectively at concentrations around 300 mg/mL! Within cells many water molecules are likely to be ordered (made relatively immobile) by crowded macromolecules and so are less able to diffuse freely. This means that many chemical changes within intracellular fluids that involve macromolecules are likely to be entropy-driven (see Chapter 12).
Piles of Coins

The concentration of proteins within extracellular fluids is also very high (e.g. 80 mg/ml). Cells suspended in these fluids can “loose solubility” and aggregate when either the total protein concentration, or the concentrations of certain proteins, exceeds certain limits. Normally red blood cells (erythrocytes) appear as flat red disks suspended in blood plasma. When the concentration of proteins in plasma is increased only slightly the red cells move, as if under the direction of unseen hands, and queue up to form “piles of coins” or “rouleaux”. It is possible to watch this and demonstrate the specificity of the aggregation by light microscopy. In mixtures of red cells from different species, cells of the same species preferentially aggregate with each other (Fig. 13-1).

![Diagram of rouleaux formation](image)

Fig. 13-1. Specificity of rouleaux formation. A rouleaugenic agent (e.g. polymerized blood albumin) is equally active in aggregating red blood cells from animal species A or from animal species B. The aggregates appear as rouleaux, or “piles of coins.” When the two cell populations are mixed and then treated with the rouleaugenic agent, each of the resulting rouleaux should either contain both A and B red cells (indicating non-specific aggregation), or all A and all B red cells (indicating specific aggregation). The latter alternative is found experimentally.
Thus, the entropy-driven aggregation shows specificity — "like" aggregating with "like." In mixtures, red cells form homoaggregates (like with like), not heteroaggregates (like with unlike). This reflects a general tendency for shared regularities in structure, and shared molecular vibrations (resonances), to promote self-self interactions between particulate entities, be they whole cells, or discrete macromolecules (see Chapter 2).

It is important to note that the aggregation is a response to an increase in total proteins in the surrounding medium and there is no direct cross-linking of cells by the proteins. The proteins are not acting as cross-linking agents (ligands; Fig. 13-2).

![Fig. 13-2. Distinction between (A) aggregation of red blood cells by an extracellular cross-linking agent (antibody) and (B) aggregation that does not involve a cross-linking agent. In (A) the disk-shaped red cells are cross-linked by Y-shaped bivalent antibodies (not drawn to scale). The bonding here is strong and the aggregates are not readily disrupted. In (B) the red cells adopt the energetically most favorable (entropy-driven) pile-of-coins conformation (rouleaux). The bonding here is weak and the aggregates are readily disrupted. Within an organism the high protein concentration of the inter-cellular environment should promote the aggregation of similar cells into a common tissue, a process that, initially, might not need cross-linking agents.](image)

The increase in protein concentration can be produced non-specifically by addition of an excess of any one of a variety of proteins. Thus, rouleaux-generation is a collective function of proteins. The appearance of rouleaux in a blood sample provides a clinical index of the underlying state of the plasma proteins, not of the red blood cells. However, if red cells themselves are thought of as merely large proteins, then the phenomenon of rouleaux formation can assist our understanding of the phenomenon of protein aggregation as it occurs in concentrated solutions. This can be of help when considering possible mechanisms for intracellular self/not-self discrimination [4].
Homoaggregates

A specific protein can be induced to "self-aggregate" so forming homoaggregates (Greek: homos = same). This is brought about either by increasing the concentration of surrounding proteins or by increasing the concentration of the protein itself. In some cases homoaggregate formation reflects an obvious physiological function. Thus, coat protein molecules of tobacco mosaic virus (TMV) naturally aggregate to form the outer coat that protects virus nucleic acid during passage from cell to cell. But if an enzyme aggregates, its activity often diminishes and it may become insoluble (i.e. it precipitates). To this extent, aggregation can be non-physiological and harmful to the cell.

On the other hand, proteins are usually unstable, with life-spans extending from minutes to days, and homoaggregation can assist protein breakdown. This is a stepwise process by which a protein first becomes tagged (marked) as ready for degradation, and then is cleaved into fragments (peptides). Finally the peptides are degraded to the relatively stable amino acid "building blocks" from which new proteins can be assembled. The continuing process of synthesis, breakdown and reassembly of proteins, is referred to as "protein turnover" (see Chapter 2).

Sometimes, however, under special circumstances to be considered later, certain peptide fragments are not degraded. Instead, they are united with peptide-display proteins (major histocompatibility complex proteins; MHC proteins), and taken to the exterior of the cell. Here they are recognized by cytotoxic lymphocytes, which destroy the cell. The underlying principle that emerged in the 1980s was amazingly simple, and I (and probably others) kicked myself for not realizing it earlier. You do not need a whole elephant to diagnose elephant. You do not need a whole protein to diagnose a protein. Immunologists already knew this! They had coined the expression "antigenic determinant" for a part of a protein (antigen) that would suffice for an extracellular immunological recognition event. Yet, no one seemed to have considered the possibility that cells might detach the equivalent of an antigenic determinant from a protein intracellularly prior to engaging in a diagnostic recognition event.

In studies with TMV, Lauffer showed that aggregation involves the liberation of water molecules bound to the macromolecules [5]. Thus, while it might appear, from the observed aggregation, that entropy was decreasing, the increase in disorder of the liberated water molecules more than compensated for the increase in order of the macromolecules. System entropy increased. If the aggregation were entropy-driven (endothermic), then it should be promoted by a small increase in temperature. Indeed, aggregation can be induced by increasing the temperature over a narrow range, much lower than would be needed to disrupt the structure of (denature) the protein (Fig.13-3).
Collective Pressure

Homoaggregation at high protein concentrations generates specific, but relatively unstable, aggregates (i.e. only weak chemical bonding is involved). If you were to progressively concentrate a mixture of proteins (A, B, C, D) in aqueous solution then, at a certain critical concentration, one of the proteins, say A, would self-aggregate. In this process each molecule of A would loose some of its bound water. In the absence of the surrounding proteins (B, C, D) much higher concentrations of A would be required for aggregation to occur. Thus, a group of proteins collectively exerts a "pressure" (due to their binding of water) tending to force individual protein species to self-aggregate and
give up their bound water. A protein species with the greatest tendency to
self-aggregate (a function of factors such as structure, molecular size, and ini­
tial concentration) aggregates first. As total protein concentration increases,
other protein species aggregate in turn.

The concept of the "crowded cytosol" implies that much intracellular wa­
ter is bound to proteins, so that there is always a strong standing pressure to
drive into homoaggregates any macromolecular species that exceeds the
solubility limits imposed by the macromolecules surrounding it. Each indi­
vidual macromolecular species can both contribute to, and be acted upon by,
the pressure. Thus, each macromolecular species can have this collective
function as well as a specific function. Both functions can affect phenotype
and hence influence selection by evolutionary forces.

Concentration Fine-Tuning

It follows that the concentration of a protein is an important attribute that is
not necessarily related, in any simple way, to the role the protein might nor­
mally play in the life of a cell. A protein has evolved to carry out a specific
primary task. On grounds of economy, it might be supposed that evolution­
ary forces would have pressed for a maximization of specific activity (e.g. en­
zyme activity/protein molecule). This would minimize the necessary concen­
tration of the protein. But there is no particular virtue in minimizing the con­
centration of a protein.

Provided its concentration is not extreme, a protein itself does not burden a
cell. Indeed, if a collective function (e.g. the ability to exert a pressure to
drive other proteins from solution) were an important attribute of a protein,
then the steady-state concentration of the protein might tend towards the
maximum compatible with the protein remaining in solution without self­
aggregation. This would tend to counteract any tendency to maximize spe­
cific activity because the number of molecules present would suffice for the
necessary level of activity, and there would be no selection pressure for them
to improve on a per-molecule basis.

The steady-state concentration of a protein is determined by evolutionary
forces acting on parts of the corresponding gene (i.e. base mutations) to af­
fect factors such as mRNA transcription rate, and mRNA and protein stabili­
ties. For example, a mutation that decreases the transcription rate of a gene
dereases the concentration of the corresponding mRNA, and hence the con­
centration of the protein that is made by translating that mRNA. A protein
within the cell ends up with a certain specific activity, which can be less than
the maximum possible. Over evolutionary time, the concentration of the pro­
tein is fine-tuned to the concentrations of its fellow travellers – the other dif­
fusible proteins with which, from generation to generation, it has shared a
common cytosol. In this circumstance, whereas normally the protein would
be soluble, a mutation in the corresponding gene could result in homoaggregation and insolubility. This might provide an opportunity to register the protein as “not-self.”

**Heteroaggregates**

Why fine-tune? In general, as hinted at in Chapter 2 with the forgery metaphor, fine-tuning creates a narrower frame of reference so broadening the range of events that may be discerned as falling outside that frame. A factor favoring the precise fine-tuning of cytosolic protein concentration would be the need to discriminate self-proteins both from mutated self-proteins and from foreign proteins (such as might be encoded by a virus). We need to discriminate between “self,” “near-self,” and “not-self.” Mutated self-proteins are only slightly changed and so can be considered as “near-self” proteins, rather than “not-self” proteins. But, although “near-self,” they need to be registered as “not-self.” Virus proteins would be foreign and less likely to correspond with self (i.e. they would be “not-self”). But viruses that could accept mutations making their proteins more like their host’s proteins (i.e. approach “near-self”) might be at a selective advantage. As we shall see, this advantage would fade if host defenses were attuned for discriminating between self and “near-self” in such a way that the latter would register as “not-self.”

As discussed in Chapter 12, a system for intracellular self/not-self discrimination could have evolved when the first unicellular organisms arose and were confronted with the first prototypic viruses. A mutated self-protein might have lost activity either as a direct result of the mutation (e.g. the mutation might have affected the active centre of an enzyme) or because the mutation had decreased solubility, perhaps manifest as homoaggregate formation. However, when mutated, a resulting structural (or vibrational) change might, by chance, have *created* some degree of reactivity with one or more of the many other diffusible protein species within the same cell. Hence, the mutated self-protein might “cross-seed” the aggregation of unrelated proteins. Heteroaggregates might form (Greek: *hetero* = different). There could then be a loss of function not only of the primarily mutated protein, but also of the coaggregated proteins. Thus, a mutation in one protein might affect the functions of other specific proteins in unpredictable ways, generating complex mutational phenotypes (pleiotropism). Certain clinical syndromes may be sets of such diverse, often not obviously related, altered phenotypic characters that are observed in disease states.

To the extent that a primary mutation does not result in heteroaggregate formation, then the function of a cell where the mutation has occurred might not be affected, and the cell might persist. Cells with mutations that result in heteroaggregate formation are more likely to be functionally impaired. Unless aggregation were required for its primary function, a protein would
have been fine-tuned over evolutionary time so as not to interact with the many thousands of other diffusible protein species with which it had been travelling through the generations in the same cytosol. Such interaction might have impaired the function both of the protein and those with which it interacted. So organisms with mutations leading to interactions would have been negatively selected.

Heteroaggregate formation could, however, be of adaptive advantage if the primary mutation was in a gene controlling cell proliferation, which might result in a loss of control and hence cancer. In this case, a gene encoding, by chance, a normal protein that would coaggregate with a mutant cancer-causing protein (oncoprotein) would confer a selective advantage, over and above that conferred by virtue of the gene’s normal function (i.e. while still retaining its normal function, it would be positively selected for encoding an “immune receptor” that would function as a detector of “near self” oncogenic changes). Genes encoding products with such coaggregating functions would tend to make cancer a disease of post-reproductive life. This is a time when selective factors that tend to promote the number and reproductive health of descendents are less important; so cancer prevention would be less evolutionarily advantageous (6).

**Protein “Immune Receptors”**

From this we see that potential functions of a protein include (i) its primary function (e.g. enzyme activity), (ii) its contribution to the total cytosolic aggregation pressure, (iii) its ability to form heteroaggregates with either mutated self-proteins (e.g. oncoproteins) or foreign proteins that may have been introduced by a virus. In the latter respect, cytosolic proteins can be regarded as “intracellular antibodies,” or protein “immune receptors” (see Fig. 12-1).

The genes of a host cell and the genes of an invading virus differ in various ways that might assist discrimination between self and not-self. Within the species-limit, host cell self-genes travelling together through the generations should have had ample opportunity collectively to coevolve and fine-tune to each other. On the other hand, the goal of a virus is to multiply and spread, preferentially within the lifetime of its host. From this it might be thought that the cytosolic concentrations of virus gene products would be less fine-tuned than the cytosolic concentrations of host gene products. However, the high replication and mutation rates of viruses relative to their hosts make it likely that viruses would be no less fine-tuned than their hosts. Indeed, host fine-tuning over evolutionary time would have tended to create a uniform intracellular environment, which would decrease a major virus anxiety – that of anticipating the conditions it would find in its next host. Thus, it is unlikely that the proteins of a “street smart” intracellular pathogen would readily exceed the solubility limits imposed by host proteins in the crowded cytosol.
However, in their role as "immune-receptors," host cytosolic proteins could form a diverse antibody-like environment capable of forming hetero-aggregates with virus proteins, which would accordingly register as not-self (see Chapter 12). This primary self/not-self discrimination event, perhaps in an environment made permissive as part of a response to dsRNA alarms (i.e. cells are alerted to become more conducive to registering not-self), should result in processing of the hetero-aggregates by cytoplasmic structures known as proteosomes. Here there is creation of protein fragments (peptides) that are not further degraded to amino acids. Instead, they are displayed at the cell surface by the peptide-display proteins (MHC proteins). This display is recognized by cytotoxic cells of the lymphoid system (T-lymphocytes), which destroy the virus-containing cell. The organism then loses the services of one of its cells. But most tissues can readily replace lost cells by the mitotic division of other cells that are not infected.

How diverse is the intracellular protein immune-receptor repertoire likely to be? At the very least it would include the many diffusible protein species normally present in the cytosol of a differentiated cell type. In addition, when a virus "tripped" the self/not-self discrimination alarm, there might be translation into proteins of some of the RNA products of the "hidden transcriptome" (see Chapter 12) [7]. These might include the products of tissuespecific genes not normally expressed in a host cell of a particular tissue type (i.e. a brain-specific gene might be abnormally expressed in an infected kidney cell).

**Phenotypic Plasticity**

It would be expected that within, say, a kidney cell, such not-normally-expressed products would be present only at the very low concentrations needed for their role in identifying the virus proteins with which they react (so registering virus proteins as not-self and tripping appropriate alarms). But there would also be the possibility, in the case of proteins normally required at very low concentrations, of an unwelcome effect on the phenotype. For example, very low concentrations of critical regulatory proteins are synthesized at various unique time-points during embryogenesis, so bringing about developmental switches. Thus, developing embryos within pregnant females undergoing virus attack (or an equivalent stress) might produce a developmental switch protein at the wrong time. Offspring would then appear with mutant phenotypes sometimes similar to the mutant phenotypes observed among the offspring of pregnant females exposed to mutagens (e.g. X-rays), which had mutated the DNA of their embryos.

However, if viable, the mutant offspring of a parent that had been under virus attack would not, in turn, be able to pass their mutant characters on to their offspring. In contrast, the mutant offspring of a parent that had been
treated with mutagens might be able to pass on their mutant characters if the mutation had affected developing germ-line cells. The mutant forms that resulted from developmental stress would be classified as “phenocopies,” rather than “genocopies,” since their mutant characters would not be genetically inherited (i.e. there would be no underlying causal genetic change) [8]. In other words, stressed organisms display “phenotypic plasticity” not “genotypic plasticity.”

**Polymorphism Individualizes**

High host polymorphism would make it difficult for viruses to anticipate the “immune receptor” RNA and “immune receptor” protein repertoires of future hosts (see Chapter 12). Furthermore, peptides generated from heteroaggregates of virus and host proteins, would include host protein-derived peptides. These self-antigens, as well as, or instead of, the antigens of the pathogen, would then serve as targets for attack by cytotoxic T cells [9]. Accordingly, the variability (polymorphism) of intracellular proteins (i.e. differing from individual to individual of the host species) would tend to individualize the immune response to intracellular pathogens (and cancer cells). T-lymphocytes from one virus host (or one cancer subject) might not recognize cells of another host infected with the same virus, or afflicted with the same type of cancer. From this perspective, protein polymorphism is not “neutral,” as sometimes supposed, but serves to adapt potential host organisms as “moving targets,” so mitigating against pathogen preadaptation.

Thus, the designation by the host of a virus protein as “not-self,” might involve both quantitative factors (i.e. homoaggregate formation if a virus protein’s concentration exceeds a solubility threshold, which the virus might easily avoid by mutation), and qualitative factors (i.e. the recruitment of various polymorphic host proteins into protein heteroaggregates, which the virus might not so easily avoid by mutation). However, the T-lymphocytes primed by specific peptide fragments from self antigens might, besides multiplying and attacking the virus-infected (or cancer) cell, also react against the same self antigens should they, perchance, be displayed by normal host cells. In cancer patients this could result in immunological diseases of various tissues, other than the primary cancer tissue [10].

An interesting example of such a “paraneoplastic disease” can arise when melanoma tumors are attacked by cytotoxic T-lymphocytes that recognize peptide fragments from melanin pigment, a normal tissue-specific product. The T-lymphocytes can react against both the tumor cells and some normal melanin pigment-forming cells, creating white skin patches (vitiligo) [11]. Under normal circumstances many intracellular proteins (potential self antigens) are not displayed by cells, so there is no deletion (negative selection) of
T-lymphocytes with the potential to react with these proteins when the T-lymphocyte repertoire is being purged and moulded (see below). Intriguingly, white skin patches are most likely to form where there has been local trauma to the skin [12]. Just as the sound in the wood alerts the sheep, which move away (see Chapter 12), so externally-inflicted stress (not-self) appears to alert skin cells, which are provoked to display fragments of self-antigens. If the concentration of the corresponding specific T-lymphocytes is sufficiently high, then the traumatized, but essentially normal, self-cells are destroyed. By the same token, a minor knock on the head that might normally pass unnoticed, could lead to an immunological disease of the brain if, at that time, cytotoxic T-lymphocytes happened to be rejecting, say, incipient kidney cancer cells that were displaying fragments from a protein that was normally brain-specific (Fig. 13-4). Thus, paradoxically, the first symptom of a kidney cancer might be neurological impairment. Indeed, the T-cell attack might eliminate the provoking cancer, which might then never be detected. Instead the subject would have acquired an autoimmune disease of the brain.

**Fig. 13-4.** Paraneoplastic disease. Normally a cytotoxic T-cell (central black ball) with a receptor that recognizes a specific MHC-associated brain peptide, will attack neither brain cells, nor cells of other tissues, such as kidney cells (*Time 1*). If the kidney cells later become kidney cancer cells (neoplastic cells), they may then display brain peptides which stimulate the proliferation of specific cytotoxic T-cells (*Time 2*). These can attack both the stimulating cancer cells and *normal* brain cells, particularly if various external stresses provoke the latter to display their own brain-specific peptides.
Death at Home or in Exile

Dividing cells are often seen when tissues are examined under the microscope. For adult organisms of relatively constant size, cell multiplication must be accompanied either by a corresponding number of cell deaths, or by the exile of superfluous cells beyond the body perimeter. Since exiled cells generally die, the options are bleak. Die at home or die in exile! The only exceptions are gametes that can find appropriate partners and so generate new individuals. If the balance (homeostasis) between cell multiplication and destruction is lost, cancer can result.

Death-style options are limited. When there is trauma to a tissue, cells may die by “necrosis,” a process that may involve activation of T-lymphocytes and the migration of phagocytic cells (Greek: phagein = to eat) from dilated blood vessels. The region may become warm, tender and red (i.e. “inflamed”). Dying cells are ingested by phagocytic cells, which degrade their macromolecules. However, usually cells are eliminated without inflammation. For example, dead skin cells are simply sloughed off into the environment. Each time you undress you discard not only clothes, but also around 400,000 skin cells [13]. Cells that cannot be discarded in this way invoke physiological auto-destructive mechanisms. Without fanfare, the cells self-digest, and their breakdown products are quietly ingested by neighboring cells (“apoptosis”). However, apoptosis is also a possible outcome of an intracellular self/not-self recognition event (see below).

The discarding of cells and/or their secretions into the environment may occur without an organism being aware of it (e.g. sloughing skin cells). However, an organism may be aware of a need to discard, and hence able to consciously control it. Thus, you blow your nose, and cut your nails and hair, at times of your choosing. In your early years, your parents assist this. Even in later years, the result may be more satisfactory if another person is involved (e.g. chiropodist, hairdresser).

At adolescence gamete production begins. In early human communities the discardment of male gametes would usually have involved a sexual partner, who would have been unaware when she was discarding a female gamete. Today, by monitoring the small change in temperature that accompanies ovulation, a human female can know when she is discarding a gamete but, in the absence of medication (e.g. for contraception), she has no conscious control over the timing. In some species, the attention of the male (e.g. visual cues) provokes ovulation.

Selfish Genes and the Menopause

Human females also cannot consciously control the time of the discardment of the uterine cells that have proliferated in anticipation of the arrival of
a fertilized ovum. In the absence of pregnancies (or medication), menstrual cycles continue until the menopause. Since natural selection generally favors those who produce most descendents, why is there a menopause? Biologists argue that human females will produce more descendents if, at around the age of 50, they discontinue gametogenesis and expend their energies in attending to the well-being of grandchildren. In other words, individuals with a "selfish" gene that, directly or indirectly, causes arrest of gametogenesis in females, have tended, in the long term, to produce more descendents (who inherit that gene and thus the same tendency for females to discontinue gametogenesis) than individuals who do not have that gene.

This evolutionary trade-off is not so apparent in males, and whether there is a male menopause (e.g. whether being a good grandfather is of greater selective advantageous than continued procreation) is a subject of debate. The discardment of male gametes is usually under conscious control. In modern communities, prior to pair-bonding (legalized as marriage), human males usually discard gametes autonomously at a time of their own choosing. This may involve artificial visual cues (e.g. female images) rather than a partner. Thus, discarded male gametes usually do not prevent menstruation or expand populations. Like other exiled cells, discarded male gametes usually die. Whereas self-discharge of gametes (like menstruation) may have been rare in primitive communities, in modern communities (like other forms of contraception) it is the norm. Yet, over evolutionary time, the shaping of our sexual biology has been mainly influenced by the norms of primitive communities. Biologists argue that "selfish" genes which, by some chain of events, cause us to frown at contraception, must inevitably have increased in human populations. Thus, for most of us the crowded planet is now a more pressing reality than the crowded cytosol.

**Molecular Chaperones**

Hosts can generate complex multigenic systems for dealing with pathogens. However, pathogens, because of their need to replicate rapidly and disseminate, usually have smaller genomes and so are less able to encode complex systems to counter the increasingly sophisticated host-systems that can arise in an escalating arms race. Large viruses (e.g. with 200 kilobase genomes) have more countermeasures at their disposal than have small viruses (e.g. with 10 kilobase genomes). So the strategy of a large virus must usually be different from that of a small virus.

The armamentarium of the host includes a set of proteins known as "molecular chaperones," which include proteins induced by stresses such as virus infection or heat shock. We came across these "heat-shock proteins" in Chapter 12. Some molecular chaperones play a normal role in cell operations, such as maintaining the structure of self-proteins in order to prevent inadvertent
aggregation. This would permit fine-tuning of concentration to approach even closer to the threshold beyond which aggregation would occur. To prevent inadvertent aggregation there would also be a certain margin for error, so that a self-protein would have to “stick its neck out,” concentration-wise, before aggregating and triggering alarms that would lead to apoptosis or peptide presentation to cytotoxic T lymphocytes. Figure 13-5 summarizes some factors influencing the normal cytosolic concentration of a diffusible protein [14, 15].

Fig. 13-5. Various factors affecting the concentration of a diffusible protein (e.g. enzyme) within the cytosol of a cell, as revealed by a hypothetical dose-response curve. The dose-response curve shows some quantitative measure of phenotype (e.g. the color of a flower) as it is affected by increasing concentrations of a gene product that generates that phenotype. For example, flower color can depend on the rate of conversion of a colorless substrate to a colored pigment product. This rate would be progressively increased by increasing the concentration of an enzyme (gene product) that catalyzes the conversion. If the concentration of gene product is directly related to the number of active gene copies (as often occurs), then the X-axis can be seen as providing an index of gene dosage (i.e. gene copy number). The phenotypic parameter (measured color) increases with gene product concentration until point A when some other factor (e.g. substrate availability) becomes rate-limiting. The curve then plateaus. Increasing the amount of gene product (enzyme) now makes no difference to the colour of the flower (i.e. there is no change in the value of Y).

In a diploid organism, B corresponds to the minimum gene dosage required to ensure that the phenotype would be unchanged in a heterozygote. The latter might have only one functional gene copy, and so there would be
half the concentration of gene product (A) as in the homozygote (B). The phenotype would still correspond to the plateau of the curve. The colour (value on the Y-axis) would be perceived as "dominant," being no less in the heterozygote than in the homozygote (i.e. there would be "haplosufficiency"). E corresponds to the concentration at which the gene product would still be soluble (i.e. the color would still be unchanged) if no other proteins were present. Above this concentration, the protein would tend to self-aggregate and the phenotypic parameter (Y-axis value) would decrease (i.e. color would fade).

D corresponds to the concentration at which aggregation would occur because of the presence of other cytosolic proteins that would promote such aggregation. The horizontal leftward-pointing arrows symbolize this aggregation pressure exerted collectively by cytosolic proteins, which tends to push the descending limb of the dose-response curve to the left. Another factor, symbolized by the rightward-pointing horizontal arrows, would be the molecular chaperones (e.g. heat-shock proteins) that act to maintain protein conformation and thus decrease aggregation (i.e. tend to push the descending limb to the right).

Thus, it would seem that the concentration of a protein in cells of different members of a species could fluctuate between points B and D. It is likely, however, that over evolutionary time genes have "fine-tuned" the concentrations of their products to a maximum consistent with avoiding self-aggregation. This point might correspond to C (marked by a vertical arrow), which is slightly to the left of D, thus providing a margin of safety against inadvertent self-aggregation.

In Figure 13-5 the leftward-pointing horizontal arrows symbolizing the pressure exerted by other cytosolic proteins that tends to reduce the solubility of a given protein. If any particular cytosolic protein mutates in a way that does not affect its concentration (e.g. it may be impaired with regard to maintaining its specific function, but not with regard to maintaining its concentration), then its ability to continue exerting this pressure is unaffected.

Molecular chaperones have the opposite effect to cytosolic proteins in general. The rightward-pointing arrows symbolize the pressure of molecular chaperones to increase the solubility of proteins. A particular type of molecular chaperone has a subset of "client" proteins. Since maintaining the solubility of proteins is a specific function of the molecular chaperones, when they mutate so that this function is compromised, then an important cellular defense against aggregation is removed. Individual protein species that might have just retained their solubility, now more readily cross the insolubility threshold. The proteins no longer have to "stick their necks out" in order to aggregate.

Proteins that are perilously close to the insolubility threshold include mutant proteins which have sustained amino acid changes that affect their conformations, but not their specific functions. As long as they can, with the help
of molecular chaperones, maintain their normal conformations, these proteins will maintain their distinctive individual functions. So no effects on the phenotypes of organisms with these mutations will be evident. However, when a molecular chaperone is mutated its client proteins now have to fend for themselves. Many proteins that are close to the insolubility threshold become more prone to aggregate. Mutant phenotypes that were previously hidden (latent) now emerge. They are "conditional mutants" – the condition being that their chaperone is not around to spruce them up. So solubilities (and hence functions) are no longer sustained. Thus, a molecular chaperone can be seen as a mutational "buffer" or "capacitor" that allows organisms to survive certain types of mutation [16].

In the latter case an underlying hidden genetic change can be revealed by chaperone malfunction. This should not be confused with the "phenocopy" phenomenon mentioned above, where an organism undergoing virus attack (or an equivalent stress) at a critical developmental stage might display mutant phenotypes, but there is no underlying genetic change [8]. The product of a gene is, because of environmental factors, expressed at the wrong time or place, but the gene itself is unmutated.

On the other hand, viral attacks change the levels and modes of expression of heat-shock proteins. If some of these do not remain in chaperone-mode, then some of the observed phenotypes might not be phenocopies, but might reflect the exposure of unbuffered mutations. The expression of these mutations by future offspring would normally also be conditional on failure of chaperone function, but there are suggestions that sometimes mutant expression can switch from conditional to unconditional, so that the expression becomes a permanent characteristic of the line (a mysterious phenomenon known as "genetic assimilation").

All this leads us to distinguish between cytosolic proteins in general, each of which has the potential to react weakly with erring members of a certain small subset of its fellow travellers [6], and molecular chaperones – professional interactors – each of which has the potential to react strongly with erring members of a certain large subset of its fellow travellers.

**Positive Repertoire Selection**

Whatever the sophistication of the defence and attack systems of a host and its viruses, the usual initiating event is one of self/not-self discrimination, be it between two RNA species or between two protein species, or be it extracellular or intracellular (see Chapter 12). Accordingly, a virus that could mutate to appear as "self" to its host should have an adaptive advantage. It would exploit the fact that during the development of lymphocytes, each specific for a particular antigen, negative selection of lymphocytes reactive with some "self" antigens had generated "holes" in the repertoire. However, dur-
ing development (e.g., creation of cytotoxic T-lymphocytes and antibody-forming B-lymphocytes) there is positive selection of those reactive with "near-self" antigens (positive repertoire selection) by mechanisms outlined elsewhere [17–20]. The development of the repertoire of cytotoxic T-lymphocytes, for example, is strongly influenced by the polymorphic MHC proteins of the host. As a virus mutates progressively closer towards the self-antigens of a potential host, the chances that the host will be immunologically prepared become greater because its lymphocytes have been positively selected to react with "near-self" MHC proteins. In other words, the virus encounters stiffened host defenses.

In this and the previous chapter we have considered immunology at the intracellular level in a way that is not found in immunology texts. For example, immunologists tend to use the term "altered self," rather than "near self." There is a subtle difference between these usages. "Altered self" implies a difference from self. "Near self" emphasizes how close an entity can come to self yet still be distinguishable from self. Immunologists also tend not to recognize the implications of the crowded cytosol for molecular interactions. But you should be warned that, while the association of peptides with MHC display proteins is well established, at this time the underlying mechanism for the association is not. Then why does a hypothetical mechanism have a place in a text on evolutionary bioinformatics?

One reason, as has been stated, is that the full understanding of the genomes of a species requires an understanding of the genomes of the species with which they have coevolved. These species interactions involve processes of self/not-self discrimination, both extracellular and intracellular. Another reason is that understanding the fundamental role of intracellular protein concentration in self/not-self discrimination can make other evolutionary phenomena intelligible. It is in such terms that we will, in the next chapter, discuss "Muller's paradox" and the mystery of sex chromosome dosage compensation.

**Summary**

The crowded cytosol is a special environment where weak interactions can be important. Here many macromolecules are close to the limits of their solubility, a condition conducive to weak, but specific, entropy-driven molecular interactions. In addition to being under evolutionary constraint to preserve the functions of their own products, genes encoding specific cytosolic proteins are also under evolutionary constraint, both to support a pressure exerted collectively by proteins to drive other proteins from solution, and to maintain the solubilities of their own proteins in the face of that collective pressure. Thus, genes whose protein products occupy a common cytosol have co-evolved such that product concentrations are fine-tuned to a maximum
consistent with avoiding self-aggregation. Cytosolic proteins collectively generate a pressure tending to drive proteins into aggregates. Each individual diffusible protein species both contributes to, and is influenced by, the pressure. Intracellular pathogens must fine-tune the concentrations of their own proteins to the solubility limits so imposed. Aggregates between viral proteins and normal host, antibody-like, “immune receptor” proteins, provide a possible basis for intracellular self/not-self discrimination at the protein level. Molecular chaperones, including heat-shock proteins, modulate this process. In such terms, we can explain Goldschmidt’s phenocopy phenomenon, and paraneoplastic diseases. During their development there is positive selection of host lymphocytes reactive with “near-self” antigens (positive repertoire selection). This counters the tendency of pathogens to mutate towards “self”. Thus, hosts whose immunological forces are poised to attack “near-self” versions of not-self (a subset of not-self), rather than not-self per se (the entire set of not-self – formidable in range), are at a selective advantage.