Early embryos of many vertebrates and invertebrates develop outside the mother and are exposed to a myriad of potential microbial colonizers. Here we discuss how these embryos are protected from microbial attacks and how they might control and shape their microbiota. In essence we delineate a new role for antimicrobial peptides both in selecting particular bacterial partners during early development and in being important components of a “be prepared” strategy providing transgenerational protection.

Introduction

“Considering the importance of the fetus to our survival as a species, it is surprising that we know so little about what protects it from microbial assault.”1 Mammalian embryos are embedded in the uterus, which provides protection during embryogenesis. Via the placenta, the mother provides oxygen and nutrients as well as embryo protection by transferring antibodies across the placenta to allow humoral immune responses early in life. Postnatally, mammals transfer antibodies via breast milk to the offspring supporting the neonate immune system.2 Birds,3 fish,4,5 amphibians6 and reptiles7 transmit passive immunity through the deposition of antibodies in eggs. In contrast to mammalian vertebrates, many other vertebrates and invertebrates release their oocytes in an environment full of microbes to develop there as “orphan” embryos. How these seemingly unprotected embryos respond to the environment-specific microbial challenge is an interesting albeit not yet understood problem. The most critical phase in the development of any embryo appears to be the period prior to maternal-zygotic transition (MZT) when the embryo starts to utilize its own transcriptional machinery.8,9 In this period the cells do not transcribe their own genes as they have only a biphasic cell cycle consisting of only two steps: the M and the S phase. Only after the MZT, when G1 and G2 phases are added to the cell cycle, embryos are able to respond actively to environmental signals for example with production of stress proteins.8 Hence, how is bacterial colonization of the early embryo controlled before MZT?

Maternal AMPs Protect the Embryo: The “Be Prepared” Strategy

For a long time now, there has been a growing awareness of vertebrate developmental biologists for the significance of the so-called “fertilization envelope” in providing microbial protection in early developmental stages. In fish, for example, the fertilization envelope shows both bactericidal activity against Vibrio anguillarum10 and antifungal action against Saprolegnia parasitica.11 This protection has to be achieved by maternal mechanisms as the early fish embryo is not using its own transcriptional machinery before MZT. Similarly, the extra-embryonic tissue of invertebrates such as the tobacco hornworm is immune competent and most likely protects the embryo from infection.12 In bumblebees, freshly laid eggs exhibit a strong antibacterial activity which is significantly increased after maternal challenge.13,14 In social insects where potential pathogens faced by the mother are also an immediate threat to the offspring this priming is of special relevance.14 While in most invertebrates the nature of the molecules involved in maternal protection are not known yet, the freshwater polyp Hydra uses maternally-encoded antimicrobial peptides of the periculin family15 to protect its embryos. In female Hydra, oocytes differentiate from clusters of interstitial stem cells committed to female gametogenesis, female interstitial cells, often referred to as nurse cells, produce AMPs of the peptide family periculin and store it in vesicles.15 Within each cluster of interstitial cells, one of the cells develops into an oocyte, while the other nurse cells are phagocytosed and become incorporated into the cytoplasm of the developing oocyte.16,18 Condensed nurse cells constitute the bulk of the ooplasm, persist throughout embryogenesis and provide active AMPs including members of the periculin family for the developing oocyte. Following fertilization, periculin containing vesicles get released to the surface of the developing embryo.

During embryogenesis the number of bacterial colonizers is increasing in number and changing in composition. For example, the bacterial phylotypes C7.1, belonging to the Pelomonas group and P1.1, representing Curvibacter sp. are present only in late developmental stages while they appear to be absent in the early embryo15 (Fig. 2). Thus, early developmental stages appear to have a microbiota which is clearly distinct from later developmental stages. Interestingly, the differential colonization is reflected in differences in antimicrobial activity in embryos compared to...
Gordon and colleagues suggested earlier that the specific structure of the microbial community associated with a given host is a result of natural selection at two levels. First, competition between members of the microbiota would exert "bottom-up" selection and second, the host level would represent a "top-down" selection on the microbial community. The innate immune system is the host's first line of contact with the microbiota and probably plays a crucial role in this "top-down" selection of the microbiota. Indirect evidence supporting this view comes mainly from the observation that defects in the host innate defense system affect bacterial colonization of the intestine. Crohn's disease and ulcerative colitis patients, for example, have abnormal composition of gastrointestinal microbes, characterized by the depletion of members of the phyla Firmicutes and Bacteroidetes, two bacterial divisions dominating the distal gut microbiota. Interestingly, patients with Crohn's disease show a reduced antibacterial activity in their intestinal mucosal extracts with strongly reduced expression of paneth cell α-defensins compared to control group of patients.

When considering the function of antimicrobial peptides from an evolutionary perspective, it may be relevant to consider that most if not all AMPs are restricted to a specific genus or even a species and are representing so-called taxonomically-restricted genes (TRGs). These and other observations make it likely, that AMPs function as host-derived regulators of microbial colonization rather than as simple killers.
Role of Associated Bacteria in Embryo Protection

Why Hydra embryos appear to select developmental stage-specific microbes is not yet understood. Are the associated beneficial microbes involved in embryo protection? Observations in a number of aquatic animals indicate a protective function of associated symbionts for the early embryo. For example, embryos of the crustacean species *Palaemon macrurus* are colonized by the symbiotic bacteria *Alteromonas sp.*, producing the secondary metabolite 2,3-indolinedione which is active against a pathogenic fungus.32 Bacteria-free embryos get quickly infected and die, whereas embryos re inoculated with *Aeromonas sp.* or treated with 2,3-indolinedione persist the infection.32 Similarly, in the salamander *Hemidactylium scutatum* a protective antifungal molecules derived from the associated bacterial community resides in the skin.33 One member of this community, the bacterial symbiont *Janthinobacterium lividum* produces two metabolites, indole-3-carboxaldehyde and violacein, which protect against infection with the fungus *Batrachochytrium dendrobatidis*.34 In addition to adult salamanders, these bacteria may protect also eggs and embryos from fungal infection since they can be passed directly from the mother to offspring in each generation and since communal nesting increases the likelihood of the transmission of the protective bacteria to the eggs.35 Preliminary observations in Hydra also point to a role associated bacteria in microbial defense since bacteria-free polyps and embryos are prone to severe fungal infection, while control animals show no evidence of fungal growth (Franenburg & Fraune S, personal observation).

Conclusions

We have discussed elsewhere the role of bacteria in animal development.36 Here we show that animal hosts at different developmental stages select for specific microbes by using distinct sets of antimicrobial peptides and that these beneficial stage-specific bacteria can have essential roles in antibacterial and antifungal defenses. However, despite the obvious and proven importance of interactions between microbes and their hosts and the fact that hosts control microbial community composition by antimicrobial peptides, little is known at present about the rules that govern the host-microbe assemblies. What are the contributions of species interactions? Is there selection at the community level, and if so, how? Equally pressing questions concern the stability and robustness of within-host microbial communities. A key point is to understand how far the overall function of the microbiota is influenced by individual as well as synergistic contributions of community members. An in-depth understanding of these points requires systematic phenotypic screens in combination with an analysis of the underlying molecular interactions, taking into account the relevant environmental variables.
Inflammation at Interfaces.

This work was supported in part by German Research Foundation (DFG) Grants (to T.C.G.B.) and German Research Foundation (DFG) Cluster of Excellence programs “The Future Ocean” and “Inflammation at Interfaces”.

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