Regulation of immunity and disease resistance by commensal microbes and chromatin modifications during zebrafish development

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AUTHOR SUMMARY

Mammals are protected during early development by maternal immunity, but how fish larvae are protected from infection before the maturation of adaptive immunity is unknown. We show here that colonization by commensals in newly hatched zebrafish primes neutrophils and induces expression of genes encoding proinflammatory and antiviral mediators. This process is mediated by the Toll-like receptor (TLR)/MyD88 signaling pathway. This process also requires modification of the histone H3 at the promoter of these genes. Together, these mechanisms protect zebrafish larvae against infection before adaptive immunity develops and prevent inflammation-associated disease during development.

A frequent assumption is that all the steps involved in the development of a complex multicellular organism are genetically preordained (1). However, the field of ecological developmental biology or "eco-devo" has just begun to focus on the idea that some developmental triggers may come from the environment (2). Particularly interesting are all the events that occur at the time of birth, when all vertebrates are subjected to an imminent colonization by diverse microbiota that inhabit the surrounding environment. Among humans living in developed countries, such a "negative" microbial influence tends to be avoided or, at least, diminished through high standards of hygiene. In contrast, research on host–microbe relationships recently has established the first evidence that early exposure to microbes is required for the maturation of immunity. This phenomenon is called "developmental immunologic programming" (DIP) (3) and describes a process whereby an environmental factor acting during a sensitive or vulnerable developmental period exerts effects that influence the structure and function of most organs, and, in some cases, will persist throughout life. This process is not limited to higher vertebrates; in lower taxa, such as fish, it is known as "bacterial priming." Therefore, DIP seems to be a conserved feature that has been preserved during evolution.

This recognition feature is particularly conspicuous in teleost fish, because they live in one of the most aggressive habitats.

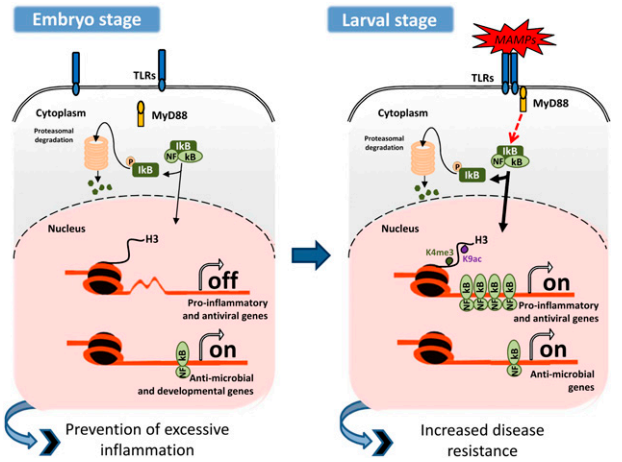

Fig. P1. Proposed models for the regulation of innate immunity during the development of zebrafish. Sensing of commensals through the TLR/MyD88/NF-κB signaling pathway in newly hatched zebrafish primes neutrophils and induces several genes encoding proinflammatory (IL-1β and TNF-α) and antiviral (interferons) mediators, which increase the resistance of larvae to viral infection. Although the promoters of genes encoding antimicrobial effectors (lysozyme and defensin), which do not have the potential to cause tissue damage, are constitutively active at high levels during development, the induction of proinflammatory genes requires the methylation and acetylation histone H3 to their promoters. This regulatory mechanism prevents the pathologies associated with excessive inflammation in early developmental stages but allows the activation of innate immunity after hatching. K4me3, trimethylated lysine 4 of H3; K9ac, acetylated lysine 9 of H3; MAMP, microbe-associated molecular patterns.

Despite potential exposure to highly inflammatory agents, fish possess specific mechanisms to discriminate between pathogens and commensals. The evidence available suggests that these processes are mediated by specialized receptors, such as the TLRs. Although beneficial, the response to either pathogens or commensals could threaten the integrity of the host if it is uncontrolled and triggers a self-directed hyperinflammation.

Here, in an effort to understand how microbial commensals are supported by their hosts, we used a germ-free zebrafish model to test the hypothesis that the contact with commensal microbiota after hatching is essential as an external environmental triggering factor to shape the development of immunity and disease resistance. We found that colonization by commensal microbes in newly hatched zebrafish primes innate immunity through TLRs and their main adaptor molecule MyD88, whose output increases the resistance of larvae to viral infection. We also observed that the induction of genes encoding proinflammatory (IL-1β and TNF-α) and antiviral (interferon) products, but not the induction of antimicrobial effector genes (lysozyme and defensin), requires the covalent modification of chromatin. These chromatin modifications occur independently of commensal microbes, although they can be regulated in their presence. In contrast, the genes encoding antimicrobial effectors, which are essential for embryos and larvae

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to defend themselves in the absence of adaptive immunity and do not possess the potential to cause tissue damage, are constitutively expressed at high levels during development, in contrast to the genes encoding proinflammatory mediators, which are transiently activated after hatching through the extensive methylation and acetylation of histone H3 at their promoters (Fig. P1). Because the signals generated by triggering the TLRs, including the transcription factor NF-κB, MAPK, and PI3K (4), are required for the regulation of important developmental genes, we propose that chromatin remodeling through covalent modification of histone H3 regulates the activation of proinflammatory and antiviral genes after hatching while preventing the pathology associated with excessive inflammation during early development. This mechanism is reminiscent of the one regulating the expression of proinflammatory (class T, tolerizeable) and antimicrobial (class NT, nontolerizeable) genes in mouse macrophages (5), and therefore we speculate that this mechanism has been conserved during the evolution of all vertebrates.

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