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Review

Antimicrobial peptide defenses against chytridiomycosis, an emerging infectious disease of amphibian populations

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Abstract

Chytridiomycosis, an emerging infectious disease (EID) of the skin caused by the chytrid fungus, *Batrachochytrium dendrobatidis*, has been linked with continuing amphibian population declines in the western USA, Central America, Europe, Africa, and Australia. Genetic analysis suggests that *B. dendrobatidis* is a recently emerged pathogen. This article reviews the biology of this pathogenic chytrid and the evidence for chytridiomycosis as a cause of declines in amphibian populations worldwide. Data are presented to show that antimicrobial peptides, produced in granular glands of the skin and released in high concentrations into skin secretions, are highly effective in inhibiting growth of *B. dendrobatidis* in vitro and may provide limited protection for some species. Ongoing studies suggest a correlation between resistance to lethal infection by *B. dendrobatidis* and synthesis of antimicrobial peptides by the host amphibian, but further research is needed to define better the role of antimicrobial peptides in protection of amphibian populations and the effect of environmental factors upon antimicrobial peptide synthesis.

Keywords: Antimicrobial peptides; Amphibians; *Batrachochytrium dendrobatidis*; Chytrid fungus; Frogs; Global population declines; Immune defense; Toads

1. Introduction

Amphibian populations have been declining worldwide at an unprecedented rate for approximately three decades. A number of excellent reviews have analyzed the possible causes of global amphibian declines [1–7]. Declines of populations and species are likely to be due to multiple interacting factors including habitat loss [6,8,9], climate change [5,10–12], introduced competitor species [6,13–17], increased ultraviolet B irradiation [4,18–22], environmental chemicals [4,23–30], and other possible causes. Here, we review the evidence implicating a chytrid fungus as the cause of an emerging infectious disease (EID) associated with recent global amphibian declines and the evidence that antimicrobial peptides in the skin secretions may provide limited protection from the lethal effects of this pathogen.

Abbreviations: EID, emerging infectious disease; MIC, minimal inhibitory concentration.

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2. The continuing problem of global amphibian population declines

Declines may best be defined as sudden population losses that are substantial and sustained. In the western USA and southern Canada, declines of the mountain yellow-legged frog (*Rana muscosa*) [31,32], montane populations of leopard frogs (*Rana pipiens*) [33,34], Cascades frogs (*Rana cascadae*) [35], foothill yellow-legged frogs (*Rana boylii*) [15], California red-legged frogs (*Rana aurora*) [15], boreal toads (*Bufo boreas*) [15], and Yosemite toads (*Bufo canorus*) [36] have occurred within the last 30 years. In Central America, the declines in the golden toad (*Bufo periglenes*) at Monteverde, Costa Rica; the harlequin frog (*Atelopus varius*) in Costa Rica and Panama, and other species at several sites in Panama in the 1980s and 1990s are also well documented [10–12,37,38]. Widespread declines in other parts of Latin America (Mexico, Guatemala, Honduras, Puerto Rico, Dominican Republic, Venezuela, Colombia, Ecuador, Peru, Brazil, and Chile), which is host to at least 50% of the world's amphibian species, have also occurred within the last 30 years [3]. Similar losses of tropical frogs in Australia during the same period have been reported [39,40]. Reports of declines of amphibian species in Latin America [41,42], Europe [43], and Africa [44] continue to emerge. These are examples of regional declines. In addition to these reports of regional declines, there have been at least four comprehensive reviews of amphibian population trends compiled from the broader amphibian population biology literature or from recent surveys. All agree that there are long term trends of declines that exceed normal population fluctuations [45–48]. Thus, it is clear that many colorful species of amphibians have suffered historically significant declines or local extinctions from which they have not yet recovered, and new population losses continue to occur.

3. The association of a chytrid fungus (*Batrachochytrium dendrobatidis*) with global amphibian declines

The evidence for a chytrid fungus, *B. dendrobatidis* [49,50], as the likely cause of some recent amphibian declines is strongest for populations in the western USA, Central America, and Australia. Individual dead frogs from multiple species collected in Panama [38] and in Queensland, Australia in 1993 and 1994 had a fungal skin infection that was later identified as a chytrid fungus [51]. Tadpoles collected at the same time exhibited partial or complete loss of keratinized mouthparts [38]. Healthy frogs exposed to skin scrapings from diseased frogs became ill or died within 18 days, whereas control frogs remained healthy. Pathogenic bacteria were isolated from less than 50% of the sick and dead frogs, and necropsy findings were not consistent with bacterial disease [51]. No viruses or protozoa were detected [51]. At about the same time, a chytrid fungus was isolated from the skin of a diseased poison dart frog and a tree frog (*Litoria caerulea*) held at the US National Zoo. This isolate was used to transmit the infection to other healthy frogs. When the newly infected frogs became sick, the fungus was again isolated from one of the infected frogs in order to fulfill Koch's postulates for chytridiomycosis and this pathogen [49,50,52]. Preserved specimens of Yosemite toads (*Bufo canorus*), leopard frogs (*R. pipiens*), and Tarahumara frogs (*Rana tarahumarae*) collected during die-offs in the California Sierra Nevada Mountains, and the Colorado Rockies during the 1970s, and in Sonora, Mexico in the 1980s have subsequently been shown to be infected with a chytrid fungus [1,53–55]. Native populations of South African clawed frogs (*Xenopus laevis*) examined in 2002 were infected with chytrids, and archived specimens suggest the infections were present in the early 1980s [56]. The most complete list of reports of amphibians infected with *B. dendrobatidis* is maintained by investigators at James Cook University, Townsville, Queensland, Australia. See [57] for website access.

4. The biology of *B. dendrobatidis*

The association of a chytrid fungus with amphibian declines and characterization of the species that is pathogenic to frogs is relatively recent [38,49–51], and many unanswered questions remain regarding our understanding of the biology and pathogenesis of this species. *B. dendrobatidis* colonizes keratinized epithelium of adult amphibians [49–51]. This ability to replicate in the outermost layer of cornified skin cells at