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How human pathogenic fungi sense and adapt to pH: the link to virulence

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The ability of fungal pathogens to cause disease is dependent on the ability to grow within the human host environment. In general, the human host environment can be considered a slightly alkaline environment, and the ability of fungi to grow at this pH is essential for pathogenesis. The Rim101 signal transduction pathway is the primary pH sensing pathway described in the pathogenic fungi, and in *Candida albicans*, it is required for a variety of diseases. As more detailed analyses have been conducted studying pathogenesis at the molecular level, it has become clear that the Rim101 pathway, and pH responses in general, play an intimate role in pathogenesis beyond simply allowing the organism to grow. Here, several recent advances into Rim101-dependent functions implicated in disease progression are discussed.

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Introduction

The ability of cells to sense and respond appropriately to environmental pH is essential for life. In fungi, adaptation to neutral-alkaline environmental pH is primarily governed by the Rim101/PacC signal transduction pathway. While important for adaptation in environmental fungi, such as *Saccharomyces cerevisiae*, and pathogenic fungi of plants, such as *Ustilago maydis*, this review focuses on the role the Rim101/PacC pathway plays in the mammalian host.

The mammalian host environment can generally be considered to be at a pH slightly greater than neutral. The pH of human blood and tissues is 7.4 ± 0.1; the pH of murine blood and tissues is 7.2 ± 0.1. However, this represents a rather limited view of the host environment from a standpoint of pH, when mucosal and other sites exposed to the outside world are considered, dramatic variations from this slightly alkaline pH are found. One obvious example is the digestive track, which shows spatial variations in pH from extremely acidic (pH < 2.0) to more alkaline (pH > 8.0). Further, temporal changes in pH within a single site have been well documented, such as within the oral cavity following the fermentation of dietary sugar by endogenous microbes [1]. The vaginal cavity is an acidic environment, pH $\sim$4; however, increases in vaginal pH occur in conjunction with menses. Thus, while fungi must be able to adapt to changes in pH within the host, most if not all pathogenic fungi must be able to thrive at neutral-alkaline pH within host tissues in order to cause disease. Here, the signaling pathways required for growth and adaptation to host pH and the contributions these pathways make to pathogenesis are addressed.

Why host pH matters to fungal pathogens?

Environmental pH has dramatic effects on cells. Proteins have a pH optima for activity and much above or below this optima, the protein is often nonfunctional. Thus, surface exposed and secreted proteins will potentially be nonfunctional in one host site but not in another. Fungi have several signaling pathways that allow them to sense environmental pH and then regulate the expression of the appropriate repertoire of genes encoding exposed proteins for the given pH of the environment.

Neutral-alkaline pH causes a number of stresses on fungi, one of the most significant is in nutrient acquisition. Most cellular nutrient uptake occurs at the plasma membrane and is driven at least indirectly by a proton gradient. As pH increases establishing a working proton gradient becomes more difficult if not impossible. Further, the solubility of essential elements, including iron, is dependent on pH. The transition between the soluble ferrous (Fe^{2+}) and insoluble ferric (Fe^{3+}) states of iron are pH-dependent, with a 1 pH unit increase promoting a 1000-fold change in the equilibrium toward the insoluble ferric form. It is worth noting that in the mammalian host environment, iron is sequestered away from microbes: being stored in intracellular ferritin complexes; the trace amounts of extracellular iron bound by transferrin in the tissues and lactoferrin on mucosal surfaces. In fact, host iron withholding processes are an important aspect of innate immunity. Thus, pathogenic fungi are iron limited owing to the effects of pH and innate immunity.

The ability of fungi to grow preferentially on acidic media has been used by clinical microbiologists for over a century to isolate fungi from the vast number of rapidly growing bacteria. However, while many pathogenic fungi are generally tolerant to acidic pH, there are stresses

associated with growth at acidic pH. For example, weak acids can readily kill microbes, including fungi. In the acidic vaginal cavity, lactate, a weak acid, is not completely dissociated. Small organic molecules, like lactate, can readily cross the plasma membrane and dissociate in the fungal cytoplasm leading to cytosolic acidification and ultimately cell death. Thus, growth at acidic pH confers stresses on cells distinct from those stresses observed at alkaline pH.

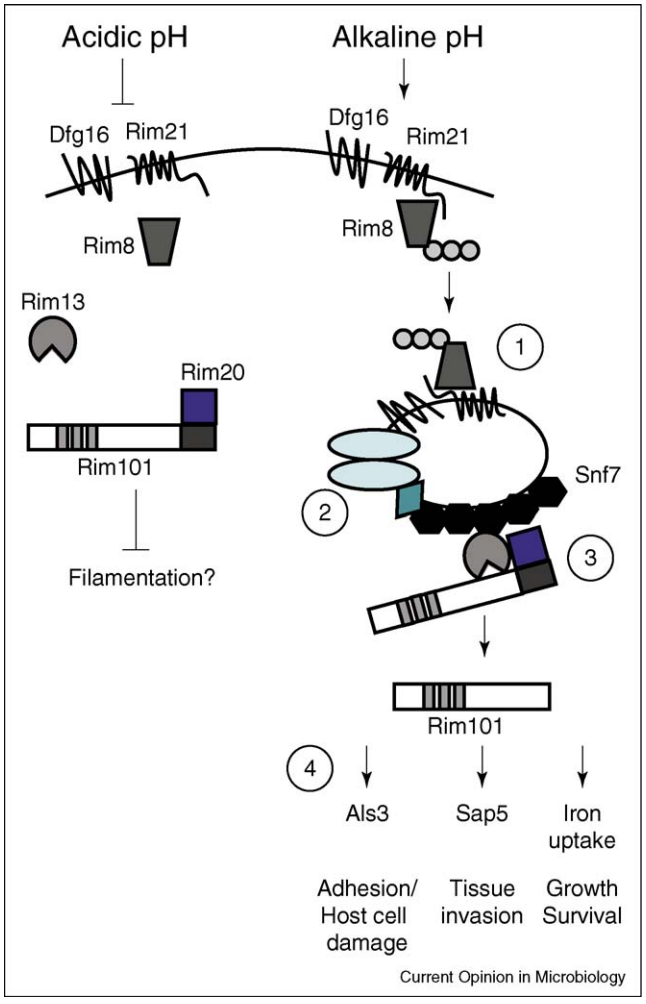
The ability to respond to environmental pH conditions is critical. However, this has been relatively understudied in the fungal pathogens. One exception to this is the analysis of Rim101-dependent neutral-alkaline pH responses.

Adaptation to neutral-alkaline pH

The responses and pathways responsible for adaptation to neutral-alkaline host environments in *Candida albicans* have received the most attention. Environmental pH has profound effects on *C. albicans* morphology with acidic pH promoting yeast cell growth, neutral-alkaline pH promoting filamentous growth. Because the morphological transition is a critical *C. albicans* virulence trait [2], early studies were conducted to identify the effectors of adaptation to environmental pH. First, it was found that the homologs *PHR1* and *PHR2*, which encode functionally redundant cell wall β-glycosidases, are pH-regulated albeit in opposite directions: *PHR1* is expressed preferentially at neutral-alkaline pH; *PHR2* is expressed preferentially at acidic pH [3,4]. Further, it was established that *PHR1* and *PHR2* are only required for virulence in host sites with a pH that match the expression patterns observed *in vitro*: *PHR1* for systemic infection; *PHR2* for vaginal infection [5•]. These seminal studies clearly established the importance of adaptation to environmental pH in the host and led to the identification of the responsible signal transduction pathways.

The Rim101 signal transduction pathway is arguably the best-studied fungal pH sensing pathway. Several recent reviews on this pathway have been published and thus, only a brief synopsis of the pathway as it is currently understood is provided here (Figure 1) [6,7]. The Rim101 pathway was first identified in *Aspergillus nidulans* (termed PacC in *Aspergillus* species) and *S. cerevisiae* and has since been found throughout the ascomycetes as well as the basidiomycetes [8–10]. Environmental pH is sensed by the plasma membrane receptor proteins Dfg16 and Rim21, of which at least Rim21 requires Rim9 for efficient localization to the plasma membrane [11–14]. The stimulation of these proteins by neutral-alkaline environmental pH triggers the ubiquitination of the Rim21-associated protein Rim8 [15•], which promotes endocytosis. This recruits the endosomal-sorting complex required for trafficking (ESCRT) protein complexes ESCRT-I, ESCRT-II, and the ESCRT-III components Vps20-Snf7 [16•]. Snf7 oligomerizes on the surface of the endosome and recruits Rim13, a calpain-like protease, and Rim20 [16•,17,18]. Rim20 binds to the C-terminal inhibitory domain of the inactive full-length transcription factor Rim101, which is recruited to the endosomal membrane [19]. Once Rim13 and Rim101 are brought in proximity via Snf7 and Rim20, proteolytic removal of the inhibitory C-terminal domain of Rim101 occurs and this processed active form of Rim101 translocates into the nucleus and governs transcriptional changes that promote neutral-alkaline pH-dependent responses [20,21,22•].

Figure 1



Current model of Rim101 pathway signal transduction and functions of this pathway in relation to pathogenesis. In acidic environments, the Rim101 pathway receptors Dfg16 and Rim21 are not stimulated. The pathway is not activated and Rim101 is not activated by Rim13-dependent proteolytic processing. While generally thought to be inactive, unprocessed full-length Rim101 may function to prevent filamentation. In neutral-alkaline environments, Dfg16 and Rim21 are stimulated, leading to the ubiquitination of Rim8, which is predicted to promote endocytosis (1). This leads to the recruitment of ESCRT-I, ESCRT-II, and Vps20-Snf7 (2). Snf7 oligomerizes and recruits Rim13 and Rim20 allowing Rim101 processing (3). This processed active Rim101 promotes transcriptional changes in genes required for growth at neutral-alkaline pH and for pathogenesis (4).