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Review

Fungal mating pheromones: Choreographing the dating game

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ABSTRACT

Pheromones are ubiquitous from bacteria to mammals – a testament to their importance in regulating inter-cellular communication. In fungal species, they play a critical role in choreographing interactions between mating partners during the program of sexual reproduction. Here, we describe how fungal pheromones are synthesized, their interactions with G protein-coupled receptors, and the signals propagated by this interaction, using *Saccharomyces cerevisiae* as a reference point. Divergence from this model system is compared amongst the ascomycetes and basidiomycetes, which reveals the wealth of information that has been gleaned from studying pheromone-driven processes across a wide spectrum of the fungal kingdom.

Contents

1. Introduction

Pheromones are diffusible 'telesensing' molecules that may be used for a variety of purposes including quorum sensing, predator detection, and mating regulation. In this review, we examine the intricacies of mating signaling in fungi, concentrating on the roles of pheromones, pheromone receptors, and related factors such as pheromone-degrading proteases, all of which are integral to regulating communication between courting partners. What defines a mating pheromone? At its most basic level, it is a secreted molecule that conveys information from one cell to another, resulting in induction of the corresponding mating response. Signaling can involve unicellular or multicellular organisms; both are present in the fungal kingdom. Historically, the hemiascomycete yeast *Saccharomyces cerevisiae* has provided an excellent model system for exploring the mechanism of pheromone signaling. *S. cerevisiae* mating occurs between two cell types, **a** and α, in a manner that is tightly regulated by secreted pheromones. *MAT***a** and *MAT*α cells secrete **a** and α pheromones, respectively, that are recognized by sex-specific receptors on the surface of the opposite cell type. We will review how these factors regulate mating in *S. cerevisiae* and discuss how this model compares to pheromone signaling in other fungal species.

2. Pheromones regulate mating in *S. cerevisiae*, the model ascomycete

Mating in *S. cerevisiae* and other ascomycetes is regulated by transcription factors encoded at the *MAT* locus. Haploid cells that

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contain the *MAT***a** locus are classified as **a** cells, and these can mate with haploid α cells that express the *MAT*α locus (for reviews see Heitman, 2007; Lee et al., 2010). *S. cerevisiae* α cells secrete α factor, a mature peptide of thirteen amino acid residues, WHWLGLKPGQPMY, that is the processed product of the *MFα1* and *MFα2* genes. The a factor is initially translated as a prepro-protein of 165 amino acids composed of three regions: a 19 amino acid signal sequence, a 64 amino acid proregion containing several glycosylation sites, and a region containing four (*MFα1*) or two (*MFα2*) repeats of the mature α factor separated by short linker sequences (see Fig. 1A). The prepro form is translocated into the endoplasmic reticulum where the signal peptide is cleaved off and extensive glycosylation of the proregion occurs (Kurjan and Herskowitz, 1982; Singh et al., 1983; Waters et al., 1988). Upon transitioning to the Golgi apparatus, these recently added carbohydrate groups are modified and three cleavage reactions occur. First, the Kex2 protease separates the α factor repeats by cleavage after conserved lysine-arginine residues. Second, Kex1 removes these two amino acids via carboxypeptidase activity. Finally, the diamino peptidase Ste13 completes the maturation process by removing excess residues at the N-terminus of the peptide (Fig. 1A) (Julius et al., 1984; Dmochowska et al., 1987; Wagner and Wolf, 1987; Caplan et al., 1991). Mature α factor then exits the cell via the classical secretary pathway.

Processed α factor is recognized by the Ste2 receptor on the surface of **a** cells, which initiates mating signaling. Despite its short length, different regions of α factor have specific roles in Ste2 activation. Thus, the C-terminus of α factor is important for the physical interaction with Ste2, while the N-terminus is more closely associated with downstream signaling events (Naider and Becker, 2004). The central region of the peptide (proline-8 and glycine-9) is thought to adopt a β-turn, which may orient the N-terminal 'signaling' and C-terminal 'binding' domains during the interaction with Ste2 (Shenbagamurthi et al., 1983; Naider and Becker, 2004). While there is tolerance for sequence changes in these regions, specific modifications can either enhance or inhibit signaling (Raths et al., 1993; Zhang et al., 1997, 1998). In either case, the affinity of α factor for its receptor does not correlate with the ability to activate downstream signaling events (Naider and Becker, 2004). In fact, modified pheromones that bind very strongly to Ste2 often perform poorly in signal transduction. These experiments illustrate two important points in pheromone function: (1) Even in short peptide sequences specialized functions are associated with different regions of the peptide. (2) The strength of the receptor-ligand interaction does not predict the strength of the induced biological signal.

The use of unmodified peptides as mating pheromones (e.g. α factor) is characteristic of the ascomycetes, including *Saccharomyces* and *Candida* species. In contrast, pheromones consisting of both peptide and lipid components are found throughout the ascomycetes and basidiomycetes (Olesnicky et al., 1999; Davidson et al., 2000; Fowler et al., 2004; Coppin et al., 2005; Kothe, 2008). *S. cerevisiae* **a** factor is representative of this class and undergoes processing that differs from that of α factor. *MF***a***1*/*MF***a***2* genes encode a propheromone of 36 or 38 amino acids, respectively (Fuller et al., 1985; Michaelis and Herskowitz, 1988). Processing of the propheromone occurs in the cytosol, beginning with modification of a cysteine residue in the conserved C-terminal motif CAAX (Anderegg et al., 1988). The cysteine residue is first farnesylated by Ram1 and Ram2 proteins that introduce a lipid moiety, followed by proteolysis of the three most C-terminal residues by Rce1 or Ste24 (Powers et al., 1986; He et al., 1991; Boyartchuk et al., 1997; Chen et al., 1997; Tam et al., 1998; Boyartchuk and Rine, 1998). Further post-translational modifications are performed by Ste14, which appends a carboxymethyl group to the cysteine residue (Hrycyna et al., 1991; Sapperstein et al., 1994). Subsequently, a two-step proteolysis of the N-terminus occurs: Ste24 cleaves off the first seven residues, then Axl1 cleaves off fourteen additional residues, although Ste24 activity does not appear to be essential (Fujimura-Kamada et al., 1997; Tam et al., 2001; Huyer et al., 2006). The final product is mature **a** factor, a twelve amino acid phospholipid exported by the Ste6 transporter (Fig. 1B).

Sex-specific pheromone expression has been demonstrated in other hemiascomycetes such as *Candida albicans*, in the basal ascomycete *Schizosaccharomyces pombe,* and in multiple filamentous ascomycetes including *Neurospora crassa, Podospora anserina, Magnaporthe grisea* and *Aspergillus nidulans* (see Fig. 2) (Zhang et al., 1998; Shen et al., 1999; Bobrowicz et al., 2002; Coppin et al., 2005; Paoletti et al., 2007). Some ascomycete species, however, show significant variations from *S. cerevisiae* pheromone processing. For example, in the filamentous ascomycete *Hypocrea jecorina* (the teleomorph of *Trichoderma reesei*), an unusual precursor form of **a** pheromone has been uncovered. The **a** propheromone exhibits features of both *S. cerevisiae* **a** and α pheromone precursors. In particular, the C-terminal CAAX motif that is associated with farnesylsation and carboxymethylation of **a** factor is present, but so are two putative cleavage sites for the α factor protease, Kex2 (Schmoll et al., 2010). The gene encoding this pheromone was demonstrated to be essential for male fertility, but Kex2 processing was apparently dispensable since deletion of putative processing sites did not block mating. The authors found similar 'hybrid' pheromones in several *Fusarium* species, leading them to classify these

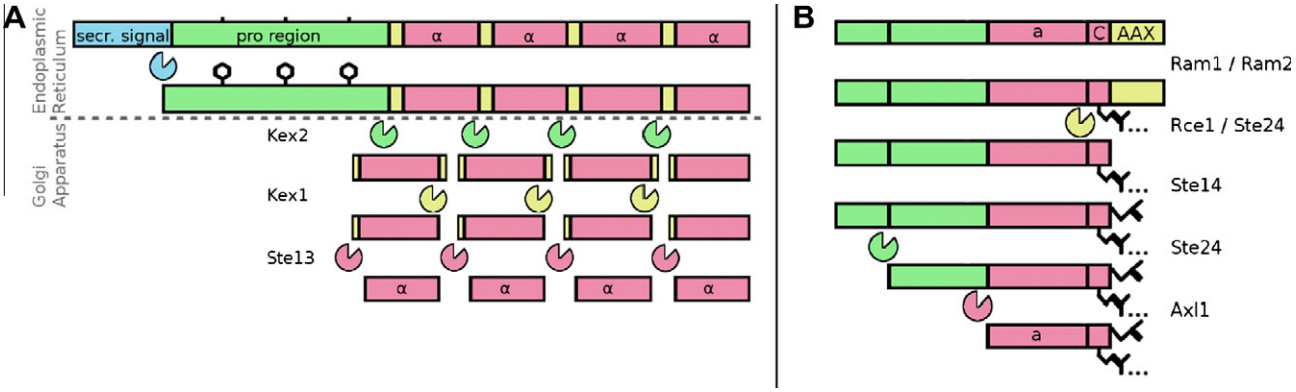

Fig. 1. Processing of *S. cerevisiae* **a** and α mating pheromones. (A). Processing of α factor: The secretion signal (blue) is cleaved to produce the α propheromone, which is glycosylated in the endoplasmic reticulum. Upon transport to the golgi apparatus, three proteolytic steps remove the proregion (green) and connecting regions (yellow) that separate copies of mature α factor (pink). (B) Processing of **a** factor: In the cytosol, **a** propheromone is farnesylated at a conserved cysteine in the C-terminal CAAX motif (C = cysteine, A = aliphatic, X = any). The amino acids (yellow) proceeding the cysteine are then removed and replaced with a carboxymethyl group. Finally, two proteolytic events remove N-terminus amino acids (green) to produce the mature **a** factor (pink).