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Mate Selection in Yeast: A Reconsideration of the Signals and the Message Encoded by them

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This paper reviews the function of peptidic pheromones in the yeast *Saccharomyces cerevisiae*. It suggests a new model, based on the handicap principle, for the mechanism that affects mate choice in yeast. The handicap principle provides a general model to interpret messages encoded in signals. According to this principle, the reliability of signals is directly related to their costs. The cost of a signal, and hence its reliability, is especially important when the signal is used to advertise mate quality. The principle suggests that low-quality individuals are unable to "pretend" to be high quality individuals because of their inability to pay the cost of a high-quality signal. Short peptides may not display the phenotypic quality of the secreting signaler because they are easy to produce. On the other hand, large proteins with post-translational modifications may vary in correlation with the variation of the phenotypic quality of the signaler. Data from various studies support our hypothesis that large proteins with post-translational modifications are involved in yeast mating. The model we suggest for yeast mating may function also in other cases, such as development processes, in which cells interact through the use of peptidic signals.

Introduction

This paper reviews the function of peptidic pheromones in the yeast *Saccharomyces cerevisiae*. It suggests a new model, based on the handicap principle, for the mechanism that affects mate choice in yeast. The handicap principle (Zahavi, 1975, 1977) provides a general model to interpret messages encoded in signals. According to this principle, the reliability of signals is directly related to their costs. The cost of a signal, and hence its reliability, is especially important when the signal is used to advertise mate quality. The principle suggests that low-quality individuals are unable to "pretend" to be high-quality individuals because of their inability to pay the cost of a high quality signal. This model is now generally accepted as the main mechanism that ensures honesty in signaling (Grafen, 1990*a*, *b*; Maynard-Smith, 1991). An important consequence of the principle is that sex-specific signals should be reinterpreted as signals that reliably display the quality of a signaling individual. Different individuals display a signal differently, thereby enabling the receiver to assess their qualities as prospective mates.

Conjugation in the yeast *Saccharomyces cerevisiae* is preceded by a courtship stage in which cells of the two mating types, **a** and α, preferentially choose sexual partners (Jackson & Hartwell, 1990*a*, *b*). **a** and α cells produce short mature peptide pheromones—**a** and α-pheromones, respectively. The pheromones serve as sex-specific signals and attract mating partners. The universal need for reliable information concerning phenotypic quality of two interacting cells requires suitable means to transfer the information. We believe that short peptides alone are unsuitable to

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advertise phenotypic qualities because they are easily expressed and thus only display genetic information. Post-translational modifications may serve to provide reliable information regarding the phenotypic quality of a signaling cell. These modifications involve usage of biochemical resources such as sugar, phosphate or lipids, and thus require a large special investment. Furthermore, synthesis of these molecules occurs in a complex series of independent enzyme systems. Post-translational modifications could therefore be an indication of both the physiological condition within the cell and the availability of biochemical resources to the signaling cell (Zahavi, 1993). Indeed, the importance of the reliability of signals in general, and especially in mate choice, has been recently recognized (Zahavi, 1981, 1987; Grafen, 1990*a*, *b*; Maynard-Smith, 1991).

The role of special investment in the signal as a means to allow discrimination between signalers with different phenotypic qualities is now well accepted in various signaling systems (Clutton-Brock & Albon, 1979: FitzGibbon & Fanshawe, 1988; Moller, 1988). Therefore, we re-examine the courtship and mating process in yeast and describe the available data which supports our conjecture that proteins, larger and more complex than the mature pheromones, are also involved in yeast mate choice.

We propose that post-translational modifications of α-pheromone propeptide are critical components for the proper selection of a mating partner. We also propose that intact α-pheromone propeptides play an essential role in the mating process and that interaction of cell bound **a**-pheromone and α-propeptides with pheromone receptors are important for partner selection and mating accomplishment. The suggested function of the yeast pheromone propeptides may be a general model for other biological systems.

Pheromone Biogenesis and Secretion

Conjugation in the yeast *Saccharomyces cerevisiae* involves fusion of haploid cells of opposite mating types, i.e. **a** and α cells (reviewed by Cross *et al.*, 1988; Herskowitz, 1989; Kurjan, 1991; Sprauge & Thorner, 1992). The **a** and α cells secrete specific peptides, **a** and α-pheromone respectively, which are essential for mating. When **a** and α cells are in physical proximity, they respond to the pheromone produced by the other mating type cell by arresting at the late G_1 stage of the cell cycle. Transduction of the pheromone signal triggers expression of several genes required for conjugation and related events.

The α-pheromone, produced by α-cells, is encoded by two loci in the yeast genome, *MF*α1 and *MF*α2. Each locus encodes for a propeptide which includes a signal sequence necessary for transport into the ER, a pro-region with three N-linked glycosylation sites, and either four (in *MF*α1) or two (in *MF*α2) tandem α-pheromone repeats (Kurjan & Herskowitz, 1982; Singh *et al.*, 1983; Kurjan, 1985) (Fig. 1). The propeptides are processed in the Golgi apparatus so that the three N-glycosylation sites on α-propeptide pro-region are modified by addition of core N-linked carbohydrates (Emter *et al.*, 1983; Julius *et al.*, 1984*a*). Three specific endoproteases encoded by the *KEX*1, *KEX*2 and *STE*13 genes, cleave the propeptide to yield mature α-pheromone peptides (also called α-factor) (Kurjan & Herskowitz, 1982; Julius *et al.*, 1983, 1984*b*; Achstetter & Wolf, 1985; Bathurst *et al.*, 1987; Cooper & Bussey, 1989; Dmochowska *et al.*, 1987; Fuller *et al.*, 1988).

The **a**-pheromone (also called **a**-factor), produced by **a** cells, is encoded by two loci, ***MFa*1** and ***MFa*2**. The **a**-propeptide does not have glycosylation sites but the carboxy terminal of the **a**-pheromone is

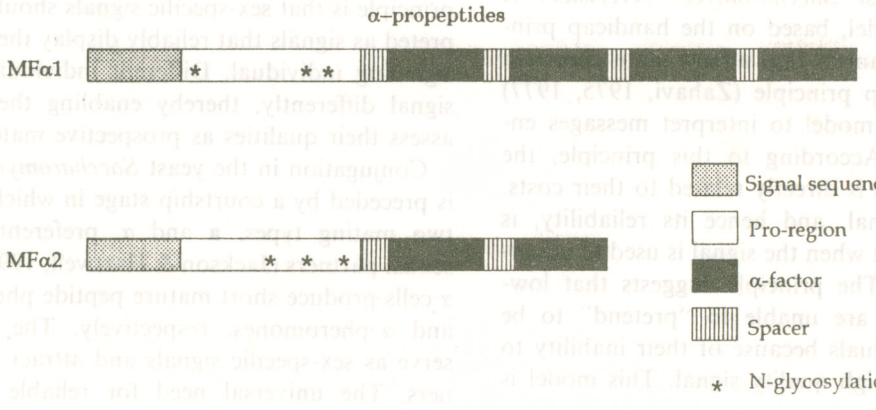

FIG. 1. The α-propeptides are encoded by two loci in the yeast genome, *MF*α1 and *MF*α2. Each locus encodes for a propeptide which includes a signal sequence, a pro-region with three N-linked glycosylation sites, and either four (in *MF*α1) or two (in *MF*α2) tandem α-pheromone repeats separated by spacer regions.

modified by addition of both a farnesyl group and methyl ester which remain on the mature pheromone (Betz *et al.*, 1987; Anderegg *et al.*, 1988). These modifications are necessary for mating, although replacement of the farnesyl group by other hydrophobic moieties or replacement of the methyl ester by an amide group still allows a low level of mating (Marcus *et al.*, 1991).

The **a** and α pheromones are secreted by two different secretion systems. The α-pheromone is processed and secreted by the typical secretory mechanism involving the Golgi apparatus (Julius *et al.*, 1984*b*). However, **a**-pheromone secretion seems to occur via a novel secretory mechanism which requires the *STE*6 gene product, an integral membrane-protein homologous to a family of ATP-dependent transport proteins (Kuchler *et al.*, 1989; McGrath & Varshavsky, 1989). This protein, which belongs to the multiple drug resistance transporters (Berkower & Michaelis, 1991), is presumed to be directly responsible for mediating the transmembrane translocation of **a**-pheromone (Berkower & Michaelis, 1991; Kuchler & Thorner, 1993).

Pheromone as a Signal in Mate Choice

Jackson and Hartwell (1990*a*, *b*) showed that a courtship stage precedes conjugation and that both mating type cells choose their sexual partners. The authors demonstrated that cells which secrete more pheromone are preferred mating partners. It is reasonable to assume that a signal which is involved with mate choice should provide reliable information concerning the phenotypic quality of the signaling cell since the reproductive success of the emerging zygote is dependent on the phenotypic quality of the mating partners.

The variation in the phenotypic quality can be due to genetic differences as well as to physiological factors such as the amount of reserve materials, cell membrane composition, cell age, etc. However, quantitative differences in the concentrations of a pheromone peptide in a mating mixture may not be an accurate way of determining differences in the phenotypic quality of single cells. Slight differences in the distances between pheromone sources and the selecting cell, as well as the possible heterogeneity of the environment, may markedly affect the quantity of the pheromone which reaches the selecting cell. As a consequence, the ability of the selecting cell to discriminate correctly between two potential partners may be disturbed.

Furthermore, production of a plain peptide is a poor means of expressing variation in the phenotypic quality of individual cells. Any living cell is capable of protein production and the demands for production of one sequence of amino acids are not markedly different from the production of another. Hence, although a gradient of pheromone concentration may show the direction of a potential partner, it is not sufficient to select the best mate from two potential partners. This mate choice can be achieved through the use of post-translational modifications as previously mentioned.

In the following, we review various data concerning the mating process in yeast which suggest that intact α-pheromone propeptides play an essential role in the mating process and, therefore, specific conformations of the propeptides and post-translational modifications may play an important role in mate choice. In addition, we propose that interaction of cell-bound **a**-pheromone with **a**-pheromone receptors is important for mating accomplishment.

Alpha-propeptide Synthesis is Necessary for Mating

Alpha cells with null mutations in both *MF*α1 and *MF*α2 loci are unable to mate (Kurjan, 1985). Surprisingly, addition of exogenous α-pheromone up to ten times (5×10^{-6} M) the physiological concentration [10^{-7}–10^{-8} M (Thorner, 1981)] does not alleviate the mating defect of these cells (Kurjan, 1985). This result suggests that either active synthesis of α-propeptide or active secretion of α-pheromone is necessary for mating. However, secretion of mature α-pheromone is not necessary for mating because addition of exogenous α-pheromone to α *kex*2 or to α *ste*13 cells (6×10^{-7} M and 6×10^{-9} M respectively) allows mating, although these cells do not secrete mature α-pheromone (Chan *et al.*, 1983). These results indicate that synthesis of α-propeptide is important for mating, raising the possibility of an unknown function for α-propeptide in the mating process.

We suggest that a certain percentage of α-propeptides remains uncleaved, is displayed on the surface of the cell and binds to α-pheromone receptors on **a**-cells. We also suggest that conformational features of α-propeptide are important for mating partner recognition and that post-translational modifications may serve to provide reliable information regarding the phenotypic quality of the signaling cell. This hypothesis may explain why physiological concentration (10^{-7}–10^{-8} M) of extracellular α-pheromone is unable to relieve the mating defect of α-cells lacking the two *MF*α genes: such cells do not produce α-propetides and therefore mating partner recognition is disturbed. (It is possible

that when a higher concentration of exogenous α-pheromone is applied, a low proportion of default mating may occur). In the case of α *kex*2 or α *ste*13 cells, which produce α-propeptides but do not release mature pheromone, α-propeptides may be displayed on the cell surface. Therefore, mating partner recognition may occur and addition of exogenous α-pheromone in near-physiological concentration, which elicits G_1 arrest and morphological alterations of **a**-cells, is sufficient to allow mating.

An example of a signaling process involving a cell-bound molecule is the antigen stimulation of T cells, which occurs only when antigens are presented together with cell surface components encoded by the major histocompatibility complex (Korenberg *et al.*, 1983). An interaction between cell-bound propeptides and their receptors, leading to signal transduction, has also been described. The precursors of transforming growth factor α (pro-TGF-α) and epidermal growth factor (EGF) are membrane bound glycosylated propeptides which interact with EFG receptors and induce cell proliferation (Brachmann *et al.*, 1989; Morczkouski *et al.*, 1989; Wong *et al.*, 1989; Ankelesaria *et al.*, 1990).

It would be interesting to examine whether overexpression of *KEX*2 or *STE*13 genes will reduce mating efficiency. Such an overexpression, although it may increase α-pheromone secretion, might decrease the level of the uncleaved intact α-propeptides displayed on the cell surface and therefore reduce mating efficiency.

a-Pheromone Synthesis is not Necessary for Mating

A different phenomenon has been demonstrated for **a**-cells. a-cells with null mutations in both *MFa***1** and *MFa***2** loci are non-maters (Michaelis & Herskowitz, 1988). In this case, addition of exogenous **a**-pheromone restores mating, although to a low level (Marcus *et al.*, 1991). On the other hand, exogenous **a**-pheromone could not restore mating of **a**-cells with a deletion of the *STE*6 gene. Such cells produce **a**-pheromone which is not secreted but is accumulated intracellularly. Since *STE*6 is an integral membrane protein involved in **a**-pheromone secretion, it raises the possibility that STE6 protein interacts with the exogenously added **a**-pheromone (Marcus *et al.*, 1991). The **a**-pheromone is a hydrophobic peptide (Anderegg *et al.*, 1988) whose hydrophobicity may keep it associated with the secreting cell (Powers *et al.*, 1986; Betz *et al.*, 1987; Jackson & Hartwell, 1990*a*). Jackson and Hartwell (1990*a*) suggested that **a**-pheromone bound to membranes of **a**-cells may be transmitted directly to α-cells through the juxtaposed cell walls. Marcus *et al.* (1991) raised the possibility that STE6 is necessary to orient **a**-factor correctly in order to facilitate mating. Michaelis & Herskowitz (1988) suggested that cell-bound **a**-factor could provide a mating signal to α-cells that is distinct from the signal to arrest growth or cause morphological alteration. It is therefore plausible that **a**-pheromone interacts with STE6 protein and that the bound form **a**-factor/STE6 has to be recognized by **a**-factor receptors on α-cells to accomplish mating.

Exploring the Importance of α-propeptide

Several observations support our hypothesis that α-propeptide may actively participate in mating. The mating efficiency was tested in an α-strain carrying a modified *MF*α1 locus which contains a *URA*3 gene (*mf*α1::*URA*3) replacing the four copies of α-pheromone (Caplan & Kurjan, 1991). Although this strain secretes α-pheromone produced from the second locus *MF*α2, its mating efficiency is ten times lower than that of an *mf*α1 *MF*α2 strain, a strain with a deletion of the entire *MF*α1. When the *URA*3 gene replaced the four copies of α-pheromone but was in the opposite orientation, it did not interfere with the expected mating efficiency of the strain (Caplan & Kurjan, 1991). Although the differences between the two modified *mf*α1::*URA*3 products are not known, these results suggest that the mating interference in the first case was due to active interference caused by the first *mf*α1::*URA*3 propeptide. Therefore, it strengthens the possibility that α-propeptide has a role in the interaction of α-cells with the mating partner.

Furthermore, it is possible that α-propeptides interact with each other. Such an interaction may provide an explanation for the distinct structure of the two yeast α-propeptides. It may answer the following questions. Why should there be two different genes (*MF*α1 and *MF*α2) that code for the same mature pheromone? Why do they have two and four tandem repeats (respectively) and why is one of these repeats slightly different from all the others while it shows identical activity as a mature pheromone? It is tempting to speculate that the two different α-propeptides form a dimer on the cell membrane. The dimer may bind to a variable number of α-factor receptors on the **a** cell. This might provide information (perhaps through variation in the binding kinetics) concerning the number of available receptors as well as information about the quality of the signaler, through its ability to produce the dimers and their post translational modifications. Interestingly, indirect data suggest a possible aggregation of α-factor receptors

(Jennesse & Spatrick, 1986; Zanolari *et al.*, 1992). This points towards a role of receptor cross-linking in the mate choice process. Such a cross-linking may be facilitated by tandem repeats more rapidly than by a high pheromone concentration.

The importance of various parts of α-propeptide for mating was explored by Caplan & Kurjan (1991). In the first series of experiments, the importance of the *MFα*1 pro-region for mating was tested. A strain which produces no α-pheromone but is supposed to produce only the *MFα*1 pro-region was constructed. If *MFα*1 pro-region is the peptide that is important for mating, exogenous α-pheromone should alleviate the mating defect of this strain (Caplan & Kurjan, 1991). However, the mating defect could not be alleviated in the presence of 5×10^{-7} M α-factor, arguing against a role for *MFα*1 pro-region in mating (Caplan & Kurjan, 1991). Although no evidence could be presented for the actual production, modification and secretion of the *MFα*1 pro-region peptide in this mutant, this observation supports our hypothesis that intact propeptide rather than the *MFα*1 pro-region peptide may play a role in mating.

Several additional observations have been interpreted by others as supporting the hypothesis that pheromone gradient is the only way by which cells choose mating partners. However, our hypothesis offers an alternative explanation for these observations which are presented below. Caplan & Kurjan (1991) studied the mating efficiency of cells possessing *MFα*1 alleles with one through four tandem α-pheromone repeats. They found that the more α-pheromone repeats the cells have, the more efficient is their mating. Therefore, they concluded that the amount of pheromone secreted is the major reason for the different mating efficiencies. However, if an intact propeptide is important for mating, it is possible that a propeptide with four α-pheromone repeats interacts better with **a**-cells than a propeptide that has less pheromone repeats. Hence, the different propeptides rather than the amount of mature α-pheromone secreted might be the reason for the different mating efficiencies observed.

Mating-efficiency experiments in which pheromone-gradient is eliminated either by excess of exogenous α-pheromone or by using α *kex*2 or α *ste*13 strains which do not secrete α-pheromone, might differentiate between these two options. Under these conditions and in the presence of exogenous α-pheromone, we speculate that a strain producing α-propeptide with four α-pheromone repeats should mate more efficiently than a strain which produces α-propeptide with fewer α-pheromone repeats.

In an additional experiment, the effect of various modifications in the *MFα*1 pro-region but not in the α-pheromone repeats was tested. Caplan & Kurjan (1991) showed that preventing N-glycosylation, either by tunicamycin treatment or by mutations of the glycosylation sites, results in intracellular accumulation of α-propeptide and reduced α-pheromone secretion. They concluded that although N-glycosylation seems to facilitate transport through the secretory pathway, it is not essential for secretion of α-pheromone and for mating. The cells with the modified *MFα*1 pro-region were able to mate at levels proportional to the levels of α-pheromone produced.

Caplan & Kurjan (1991) suggest that these results reinforce their previous hypothesis that the only role of the α-propeptide in mating is to produce α-pheromone. However, the fact that cells with modified *MFα*1 genes produce a relatively small amount of pheromone and mate at levels proportional to the amount of pheromone secreted does not rule out an additional role for the α-propeptide. First, since the mutants show intracellular accumulation of the modified α-propeptide, the amount of α-propeptide that reaches the cell surface may be proportional to the amount of mature pheromone secreted in these mutants. The different mating efficiencies may therefore be due to different amounts of intact α-propeptides that reach the cell surface, as well as due to the different amount of pheromone secreted. Second, it is possible that choice experiments such as those presented by Jackson & Hartwell (1990*a*, *b*) would be more appropriate for testing the correlation between the amount of pheromone secreted and mating efficiency of the various *MFα*1 modified cells. Such choice experiments may show that these cells are poor mating partners to a more dramatic extent than is expected from their relative ability to produce mature α-pheromone.

The hypothesis that interaction between cell-bound pheromone and propheromone and pheromone receptors is necessary for mating also offers an explanation for the observation that excess of exogenously added **a** or α-pheromone reduces mating efficiency of wild-type cells (Sena *et al.*, 1973; Marcus *et al.*, 1991). Marcus *et al.* (1991) suggest that exogenously added **a**-pheromone distracts the pheromone gradient which is necessary for successful courtship. Alternatively, it is possible that an excess of exogenous pheromones occupies the pheromone receptors and interferes with the interaction of cell-bound pheromones and pheromone-receptors, hence reducing mating efficiency. This hypothesis may also explain why haploid cells which simultaneously produce both pheromones and pheromone receptors cannot mate (Bender & Sprauge, 1989). Perhaps the pheromone receptors on

these cells are constitutively occupied by the autocrine pheromones and the cells are unable to interact with cell-bound pheromones or propheromones on other cells and therefore are unable to mate.

How is Homosexuality Prevented in Yeast?

An additional phenomenon can also be explained by our hypothesis. When **a** and α-cells are mixed, mating happens only between **a** and α cells and not between cells of the same mating type. Both mating type cells in the mixture are arrested at G_1 and express mating genes including agglutinins which facilitate physical contact between the cells (reviewed by Cross *et al.*, 1988; Herskowitz, 1989; Kurjan, 1991; Sprauge & Thorner, 1992). Although **a** and α-cells express mating type-specific agglutinins, agglutinin expression is not essential for mating (Lipke *et al.*, 1989; Roy *et al.*, 1991). Moreover, Bender & Sprauge (1989) found that production of pheromones and pheromone-receptors is sufficient to determine mating type specificity.

No other mating type specific genes have been found, except for the genes which are involved in pheromone production, processing and secretion (Bender & Sprauge, 1989). Therefore, if unbound secreted pheromones are eliciting the mating process, it is not clear how mating between cells of the same mating type is prevented. Bender & Sprauge (1989) suggest that direct cell surface interaction between mating partners, mediated by spatially oriented pheromones and receptors, may be responsible for mating specificity. We suggest that interaction between pheromone-receptors and cell-bound **a**-pheromone or α-propeptides is necessary for mating to be accomplished. Such an interaction is possible only between cells of opposite mating type, hence preventing mating between cells of the same mating type.

Conclusions

The above examination of the mating process in yeast offers a new interpretation of the mechanism by which yeast cells may assess the phenotypic qualities of their mates. The mature short pheromone is needed for long distance communication, advertising the existence of a potential mate and its general direction. However, we propose that the final assessment which determines mating involves intact α-propeptides that are bound to the cell membrane and interact with pheromone receptors on the mating partner. We also propose that possible conformational features of the propeptide, including the post-translational modifications, provide reliable information on the cell quality: its physiological state and the availability of reserve materials. It is therefore interesting to note that the two pheromones are secreted via two different secretory pathways; the α-pheromone is secreted via the Golgi apparatus, while the **a**-pheromone is secreted via a novel secretory pathway involving STE6 which is a transmembrane protein essential for mating. Hence, when yeast cells respond to properly modified propeptides and pheromones, they increase the chance of responding to mating partners of complementary phenotypic qualities to the benefit of the emerging zygote.

The suggestion that α-propeptides are involved in the transfer of information may provide an explanation for the different structures of the α-propeptides in yeast, namely the coexistence of two different genes that produce two different propeptides while producing the same mature pheromone. We speculate that the two propeptides interact with each other on the cell membrane and bind to a variable number of receptors on the mating partner, providing reliable information to both cells. This model, although highly speculative, suggests an explanation for how yeast cells identify a mate of the other sex in an environment flooded with mature pheromones.

The transfer of signals among cells that are either very similar or even genetically identical, may involve information concerning the phenotypic situation of individual cells. We therefore propose that the mechanism suggested here for cell–cell communication in yeast may have a general importance in communication between cells. In fact, in some other cases (such as EGF) it has been established that cell bound propeptides interact with the same receptors as the mature secreted protein. We therefore believe that similar mechanisms may function in other cases in which phenotypic qualities are signaled through peptides.

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