Structure of Genes Encoding Precursors of the Yeast Peptide Mating Pheromone *a*-Factor

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The mating process between cells of the two haploid cell types of the yeast *Saccharomyces cerevisiae* provides a model system for the study of hormonally mediated intercellular interactions in a simple eukaryotic organism. The early events in the yeast mating process appear to be triggered by diffusible peptide pheromones, *a*-factor and α-factor, secreted by *a* cells and α cells, respectively (Thorner 1981).

These peptides would be expected, like other small secreted peptides, to be processed from larger precursor molecules (Herbert and Uhler 1982). This has been confirmed in the case of α-factor by analysis of the α-factor structural genes (Kurjan and Herskowitz 1982; Singh et al. 1983) and of the protein products (Brake et al. 1983; Julius et al. 1983, 1984a,b). The major α-factor structural gene encodes a primary translation product of 165 amino acids containing four mature α-factor-coding regions, each preceded by a short spacer peptide. Processing of the prepro-α-factor requires N-linked glycosylation of three sites in the leader region, proteolytic processing at Lys-Arg sequences in each spacer region by the product of the *KEX2* gene, and proteolytic maturation at the amino terminus of each resulting peptide by dipeptidyl aminopeptidase A (the product of the *STE13* gene) and at the carboxyl terminus by a carboxypeptidase-B-like enzyme.

The secretory mechanism for *a*-factor, on the other hand, has remained completely unknown. Betz et al. (1981) have made a preliminary report of the amino acid sequences of a group of four *a*-factor-associated undecapeptides of the following structure:

Protein Transport and Secretion, M.J. Gething, ed.,
Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1985.

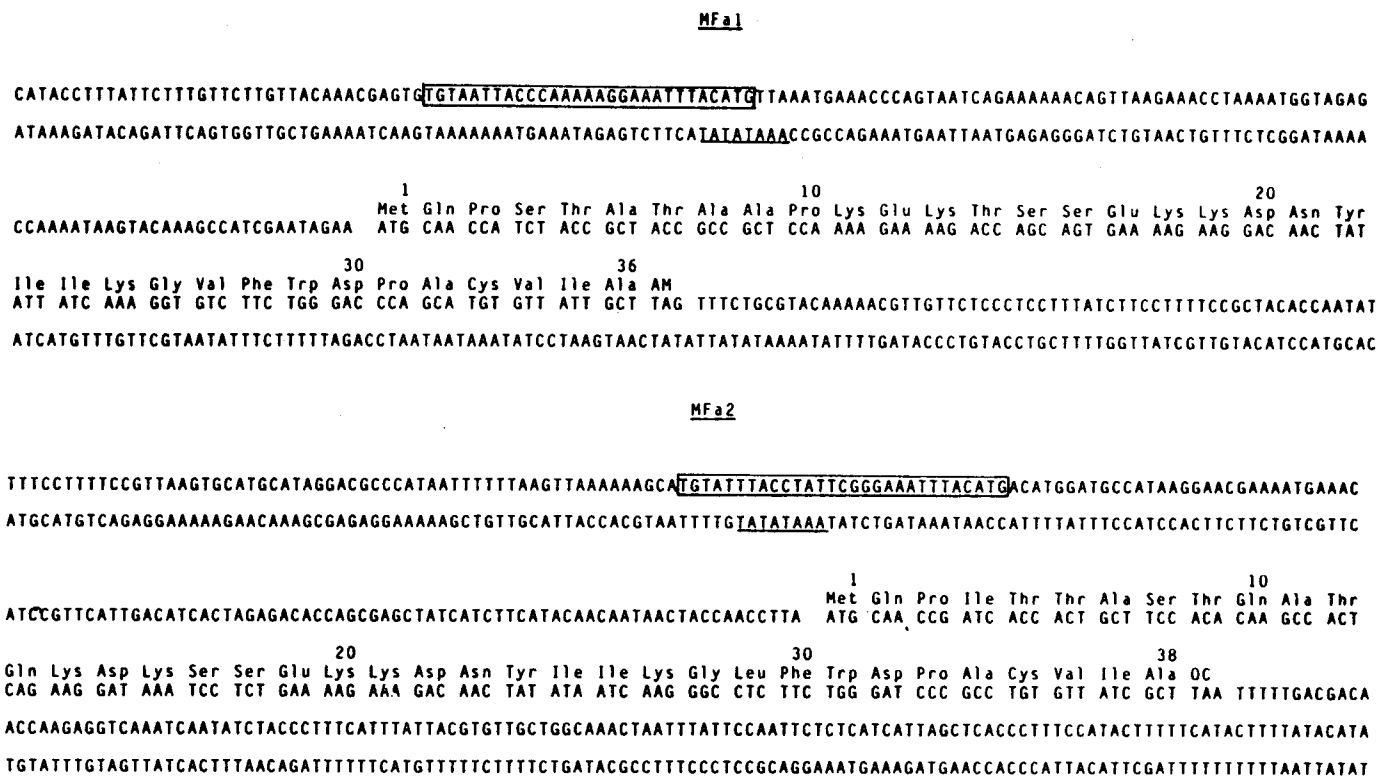

Figure 1 *(See facing page for legend.)*

There has been no report of any evidence for *a*-factor precursor polypeptides. To analyze the processing and secretion pathway of *a*-factor, we have isolated and sequenced two genes encoding putative *a*-factor precursors.

Isolation of Two *a*-Factor Structural Genes *MFa1* and *MFa2*

The approach used to isolate the structural gene(s) of *a*-factor involved hybridization screening of a yeast genomic DNA library using as probes pools of oligonucleotides complementary to DNA sequences deduced from the reported *a*-factor peptide sequences to screen bacterial colonies from a plasmid library containing yeast genomic DNA inserted into the vector YEp13 (Nasmyth and Tatchell 1980). Screening of this library resulted in the isolation of plasmids containing two different segments of yeast chromosomal DNA. These putative *a*-factor structural genes were localized to a 1.6-kb *Eco*RI-*Xba*I fragment (*MFa1*) and to a 1.6-kb *Eco*RI fragment (*MFa2*).

Prior to DNA sequence analysis of these fragments, two experiments were carried out, and the results indicated that both of these fragments contained functional *a*-factor genes. First, each fragment was used as a hybridization probe in Northern blot experiments to analyze RNAs from strains of the three different mating types. Both probes were found to hybridize to transcripts (340 bases for *MFa1* and 420 bases for *MFa2*) present in *a* cells, but not in α or diploid cells. This is a property expected of *a*-specific gene products such as *a*-factor (Herskowitz and Oshima 1981). Each fragment was also subcloned into a high-copy-number yeast plasmid vector and used to transform a *MATa* strain. The resulting transformants were tested for *a*-factor production in a replica plating biological assay (Chan et al. 1983; Julius et al. 1983). In such assays, transformants containing plasmids with either *MFa1* or *MFa2* were found to produce larger amounts of biologically active *a*-factor than control transformants carrying the vector alone.

DNA sequence analysis of both fragments (Fig. 1) revealed the presence of regions encoding *a*-factor-related peptide sequences. The deduced peptides appear to be extremely short (36 or 38 amino

Figure 1 *a*-Factor structural genes. DNA sequences of segments of yeast containing the *MFa1* (*top*) and *MFa2* (*bottom*) genes showing the deduced peptide precursor sequences. The presumed TATA box sequences are underlined. Boxed sequences are regions showing strong homology between these two genes and with other *a*-specific genes (A. Miller and K. Nasmyth, pers. comm.).

acids) precursors that contain additional amino acids at both the amino- and carboxyterminal ends. However, the aminoterminal extensions do not contain hydrophobic "signal" sequences usually found in the precursors of secreted proteins. In addition, there appears to be a discrepancy between these sequences and those in the carboxyterminal region of the reported peptide sequences. The reported amino acid polymorphism (valine or leucine at residue 6) can be explained by the deduced amino acid sequences of *MFa1* and *MFa2*.

Processing of *a*-Factor Precursors

Comparison of the two putative precursors (Fig. 2) shows a high degree of similarity throughout their peptide sequences. Both have identical carboxyterminal extensions and show the same discrepancy with the reported peptide sequences.

The peptides encoded by *MFa1* and *MFa2* both contain Lys-Lys sequences in their aminoterminal regions, which may provide a proteolytic processing site similar to the dibasic processing site found in prepro-α-factor and other peptide hormone precursors. This processing step would probably be performed by an enzyme other than the *KEX2*-encoded protease, which performs a similar cleavage in α-factor, since *kex2* mutants are not defective for *a*-factor production. Also, if cleavage of the *a*-factor precursors occurred at these Lys-Lys sites, an Asp-Asn sequence would remain; the required maturation of the aminoterminal region of molecules thus produced would apparently not be carried out by dipeptidyl aminopeptidase A, since *ste13* mutants are not defective for *a*-factor

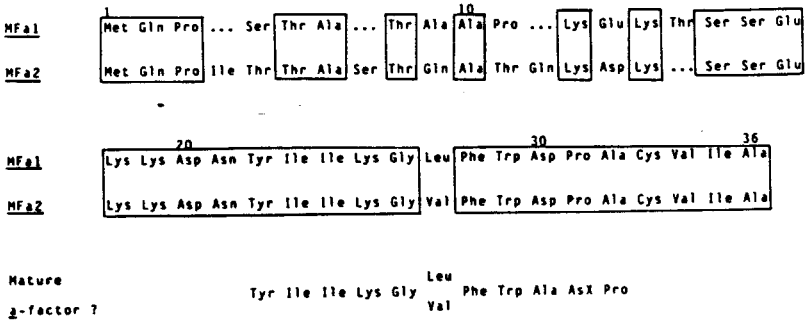

Figure 2 Comparison of the proposed *a*-factor precursor sequences. Boxed areas indicate identical residues and dotted lines indicate deletions. Also shown is the reported mature peptide sequence (Betz et al. 1981).

production. Alternatively, incompletely processed forms of *a*-factor may still be biologically active.

What gene products are required for processing and secretion of *a*-factor? Two candidates are the products of the *STE6* and *STE14* genes, since mutations in either of these genes result in defects in *a*-factor production but have no effect on α-factor production (Chan et al. 1983). To address this possibility, we used the *MFa1* and *MFa2* genes as probes in Northern blot experiments to determine whether these genes are transcribed in *ste6* and *ste14* mutants. The conclusion from these hybridizations is that both *MFa1* and *MFa2* are transcribed normally in both *ste6* and *ste14* mutants. Thus, the products of the *STE6* and *STE14* genes act posttranscriptionally and therefore may be involved in posttranslational modification of *a*-factor precursors.

DISCUSSION

The determination of sequences of the putative *a*-factor precursors described here is a useful first step in the understanding of the processing and secretion mechanism for *a*-factor. These sequences have provided the surprising result that the structure of the *a*-factor precursor is quite different from that of the precursor of the other yeast peptide pheromone, α-factor, and therefore is probably processed by a quite different pathway. The most distinctive features of the *a*-factor precursors are their small size and lack of hydrophobic signal sequence, which raises the interesting possibility that they are transported to the endoplasmic reticulum posttranslationally, rather than the usual cotranslational insertion involving the signal recognition particle and its receptor (Walter et al. 1984).

Another interesting possibility involving posttranslational modification of *a*-factor precursors is suggested by the presence of cysteine residues in their carboxyterminal extensions. Several species of Basidiomycetes yeast secrete peptide pheromones that have farnesyl moieties conjugated to carboxyterminal cysteine residues (Sakagami et al. 1981).

A thorough understanding of the secretion pathway of *a*-factor will require the use of *a*-factor-specific antibodies, which can be used to follow the fate of *a*-factor precursors in secretory mutants (Novick et al. 1981) and in mutants specifically defective in *a*-factor production.

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