Case Study IV
Mating-Type Pheromone Response Pathway of *Saccharomyces*

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**ARTICLE 1**


Haploid *Saccharomyces* cells may be either mating type a or mating type α and mating type is determined by the *MAT* locus which maps to chromosome III. There are two alleles of *MAT*: *MAT*a is present in cells of mating type a and *MAT*a is present in cells of mating type α. The *MAT* alleles encode different DNA-binding transcription regulators that control the expression of a large battery of unlinked genes involved in cell type determination (a mating type, α mating type, a/α diploid). Haploid cells mate with cells of the opposite mating type but do not undergo meiosis or form spores. Diploid a/α cells undergo meiosis and sporulate under the appropriate conditions but do not mate with any cell type. Mating and sporulation are both very complex processes and require dozens of gene functions.

This Case Study focuses on mating and, more specifically, how haploid cells sense and respond to the presence of the mating pheromone produced by cells of the opposite mating type.

Haploid cells respond to the presence of cells of the opposite mating type as follows. First, both partners in the mating pair arrest as unbudded cells in G1 of the cell cycle at Start. Start is the point at which the cell becomes committed to division and is the execution point of Cdc28 cyclin-dependent kinase action. Second, both partners form a mating projection directed toward the mating partner. Cells with this projection are pear-shaped and are quite distinct from budding cells. The shape is referred to as a schmoo. Third, gene expression of a number of mating-specific genes is induced. One of the first of these genes to be identified was a-agglutinin, a surface protein involved in adhesion to the mating partner and produced by a mating type cells. It localizes to the schmoo tip. Most strains of mating type α produce α-agglutinin constitutively but in some strains α-agglutinin expression is induced by the presence of mating type a cells.

At the time that Article 1 was published mating type α cells were known to produce a secreted peptide hormone, or pheromone, called α-factor. Similarly, in Article 1 the authors demonstrate that mating type a cells produce a diffusible factor called a-factor that was later shown to also be a peptide hormone. Thus the mating response described above is likely to be the result of events initiated by the effects of these pheromones on cells of the opposite mating type. Mutations affecting the ability of haploid cells to mate could identify genes encoding components of the pheromone sensing pathway as well as functions required for pheromone production or the process of mating itself. In Article 1, the authors develop a strategy for isolating mutants that are unable to mate, which they call 'sterile' or ste mutants.
1. Using the table below, summarize and compare the phenotypes of a mating
type, \( \alpha \) mating type, and \( a/\alpha \) diploid cells.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>a Mating-type cells</th>
<th>( \alpha ) Mating-type cells</th>
<th>( a/\alpha ) Diploid cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell cycle arrest in response to a-factor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell cycle arrest in response to ( \alpha )-factor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a-Factor secretion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Factor secretion</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Schmoo formation in response to a-factor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schmoo formation in response to ( \alpha )-factor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a-Agglutinin synthesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Agglutinin synthesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sporulation under starvation conditions</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. The \( CAN1 \) gene encodes arginine permease. Canavanine is an analogue of the
amino acid arginine and is utilized by \( Saccharomyces \) in place of arginine for
protein synthesis. When this occurs it is toxic to the cells. Based on this
description, explain the fact that recessive mutations in \( CAN1 \) are resistant to
the toxic effects of canavanine.

3. Explain the strategy behind the procedure used here to isolate nonmating
clones.

4. Are these \( ste \) mutants spontaneous or induced? Explain.

5. Is this a selection or a screen? Explain.

6. Explain why the \( can1 \) mutation must be in the strain that is mutagenized and
not in the strain to which it is mated during the mutant isolation procedure.

7. Explain why it is necessary to screen the canavanine resistant potential \( ste \)
mutant clones obtained for the following criteria.
   (a) The nutritional requirements of the potential mutant are the same as the
mutagenized parental strain.
(b) The potential mutant is unable to mate with tester strains of both mating types.
(c) The potential mutant is unable to sporulate.

8. Explain why the mutants obtained from one isolation procedure are unlikely to be independent, i.e. different mutations that arose separately.

9. List the other phenotypes for which the ste mutants were tested.

ARTICLE 2


In Article 1, MacKay & Manney isolated a series of sterile mutants that they placed into 16 different phenotypic classes. Most of these mutants were not conditional, i.e. they were sterile under all growth conditions. Thus, genetic analysis of these mutants would be difficult. Mating frequency was very low and some diploids did not sporulate. The greatest difficulty was encountered when the mutations were to be placed into complementation groups. It was not possible to do the traditional complementation test because the phenotype, ability to mate, is a characteristic of haploid cells and complementation tests are done in the diploid. So the authors did the next best thing, they mapped the mutations. If two mutations are unlinked, then they must be in different genes. If two mutations are tightly linked, then they may be in the same gene.

1. Ten of the mutants isolated in the MATα strain exhibit tight linkage to the mating-type locus and are probably alterations in MATα, which the authors refer to as MT or STE1. Diagram a cross of a Class 1 mutant (matα/ste1) to a wild-type MATα/STE1 strain. Give the genotype and phenotype of the tetrads resulting from this cross (remember the ste mutation is tightly linked to MATα). Are your results consistent with those for mutant VC2 shown in Table 2? Discuss why the authors state that, 'the upper limit for the map distance between the ste mutation in VC2 and MT is 1.2 centimorgans'.

2. Class 4 mutants were isolated in a MATα strain. The five mutants in this class are unlinked to MATα and are α-specific. That is, only MATα strains exhibit the ste phenotype. The authors refer to these mutants as ste3 mutants. Diagram the cross between a Class 4 mutant (MATα ste3) and a wild-type MATα STE3 strain. Give the genotype and phenotype (mating type and ability to mate) of the spores in the tetrad types that result from such a cross. Are your projected results consistent with those shown in Table 2 for mutant VC3? Discuss. Include a calculation demonstrating that MATα and ste3 mutations in VC3 are unlinked.
3. The results in Table 5 indicate that all five of the Class 4 α-specific mutants are tightly linked. Discuss these results and the authors’ conclusion that all the Class 4 mutations are likely to be allelic.

4. Class 5 mutants were nonspecific for mating type. That is, both a and α mating-type strains carrying these ste mutations were sterile. Four mutants fell into Class 5. Based on the results shown in Table 6, the authors place these four mutants into two groups representing two ste genes, ste4 and ste5. Discuss their reasoning.

5. Discuss the authors’ conclusion that STE2 and STE3 encode the α-factor and a-factor receptors, respectively.

ARTICLE 3


In this article Hartwell uses the G1 arrest response to isolate mutants in the pheromone response pathway. Since α-factor is easily purified from the culture medium of mating type α cells, Hartwell was able to add the pheromone directly to the culture medium. Hartwell avoided the problems in genetic analysis encountered by MacKay & Manney as follows. First, he isolated temperature-sensitive mutants. In this way, crosses could be carried out at the permissive temperature at which even the mutants were able to mate but phenotype testing could be done at the non-permissive temperature. Second, he used a genetic manipulation method to convert the MATα/MATα diploids, in which the pheromone sensitivity of the ste mutations could not be determined, to MATα/MATα homozygous diploids. Despite the fact that these strains are diploid for all chromosomes and at all loci, they are mating type a because they are homozygous for the MATα allele and they exhibit the same phenotypes as haploid MATα strains. They arrest as unbudded cells in the presence of α-factor, they schmoo in the presence of α-factor, they mate with mating type a strains, etc. MATα/MATα diploids heterozygous for different ste mutations thus can be tested for all of these mating type a phenotypes and therefore can be used for complementation analysis.

The conversion of MATα/MATα to MATα/MATα diploids relies on a type of gene conversion event or ‘loss of heterozygosity’ that occurs at low frequency at any site in the genome but perhaps is more common at the MAT locus because of the mating-type switching process that occurs in this region. The conversion of the MATα locus to MATα involves a rather large section of the chromosome and clearly extends beyond the MAT locus to the linked CRY1 gene. Hartwell cleverly uses the recessive cryl cryptopleurine resistant mutation to select for this ‘loss of heterozygosity’ event. The MATα CRY1/MATα cryl diploid strain is sensitive to cryptopleurine but, if a conversion event occurs in this region that replaces the CRY1 sequence with the cryl sequence from the homologous chromosome, then one can select these cells based on their resistance to cryptopleurine. Because of the
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linkage of \textit{MAT}_{\alpha} to\textit{cry}l, these cells are frequently simultaneously converted from \textit{MAT}_{\alpha} to \textit{MAT}_{\alpha}.

1. Describe the method used to isolate mutants resistant to \(\alpha\)-factor arrest. Include in your answer:
   (a) List the complete genotype and phenotype of the parent strain 381G. Discuss the \textit{SUP4-3} mutant allele.
   (b) Are these spontaneous or induced mutants and why?
   (c) Is this a selection or a screen and why?
   (d) Describe the conditions of this selection/screen.
   (e) Define the conditional nature of the phenotype exhibited by the mutants and describe the permissive and nonpermissive conditions.
   (f) How does the author ensure that the mutants are independent?

2. After the primary selection/screening of mutant clones, secondary testing was used. Describe this test and the desired phenotype. Give one reason why this secondary testing was carried out.

3. Before complementation analysis could be carried out, the author needed to demonstrate that each mutant clone contained only a single mutation. Describe the cross you would do to demonstrate this. Give the genotype and phenotype of the parents and the results of tetrad analysis of this diploid if the mutant contains a single alteration in a \textit{ste} gene that is not mating-type specific.

4. Before complementation analysis could be carried out, the author needed to demonstrate that the mutation in each mutant clone was recessive. Describe a method Hartwell might have used to determine this. Base your answer on the methods presented in Figure 1.

5. Describe in detail the method for complementation analysis summarized in Figure 1.
   (a) List the complete genotype and phenotype of the 381G mutant parent, the 382-31 parent, and the diploid produced by mating these two strains.
   (b) Describe the conditions for mating these strains and for the selection of diploids.
   (c) Describe the conditions for selecting \textit{MAT}_{\alpha} \textit{cry}l/\textit{MAT}_{\alpha} \textit{cry}l homozygotes.
   (d) Describe how you would determine whether the two \textit{ste} mutations in your cross are or are not complementing.

6. Hartwell (1980) tested his \textit{ste} mutants for the following phenotypes: mating ability, biochemical synthesis of macromolecules, schmoo formation, budding patterns, agglutination, and mating factor production and destruction. List the different phenotypes of mutations in \textit{ste}2, 4, 5, 7, 8, 9, 11, and 12. Why are \textit{ste}8 and \textit{ste}9 mutants considered different from the rest? \textit{SIR}3 and \textit{SIR}4 encode proteins required for silencing the normally repressed copies of \textit{MAT}_{\alpha} and \textit{MAT}_{\alpha}, called \textit{HMR}_{\alpha} and \textit{HML}_{\alpha}, located near the telomeres of chromosome
III. Explain why mutations in sir3 or sir4 would give the demonstrated phenotypes of ste8 and ste9 mutations.

7. In Article 3, Hartwell (1980) identified mutations in STE2, 4, and 5 that were also identified by MacKay & Manney (Article 2). None was isolated in STE3. Explain.

ARTICLE 4


In Article 2 MacKay & Manney suggest that STE2 and STE3 encode components of the pheromone receptors, a-factor receptor and α-factor receptor, respectively, because mutations in these genes are specific to one mating type. None of the other ste mutants was mating-type specific. Strains of the a mating type respond specifically to α-factor and thus must synthesize a receptor capable of recognizing this peptide pheromone. STE2 is a mating-type specific and temperature-sensitive ste2 mutant strains exhibit temperature-sensitive α-factor binding (Jenness et al. 1984). In Article 4, the authors clone STE2 and the deduced sequence of the encoded protein is consistent with a membrane receptor function for Ste2 protein.

1. Describe the library used to clone STE2.
   (a) What type of library was used (genomic, cDNA, expression, etc.)?
   (b) What was the STE2 genotype of the strain that was the source of the DNA fragments (STE2 or ste2)? You should be able to deduce this without reading the source reference.
   (c) Describe the vector used. Give its name and average copy number in Saccharomyces. (This vector carries LEU2 as the selection marker.)
   (d) Describe how the yeast DNA fragments were obtained and inserted into the vector.

2. Describe the cloning method.
   (a) Give the complete genotype of the Saccharomyces strain used as the host strain for the library. What specific mutant allele of STE2 does it contain and describe the phenotype?
   (b) Why were Leu+ transformants selected?
   (c) Why were transformants screened for their ability to mate at 34°C?
   (d) Why is this method referred to as ‘cloning by complementation’?

3. Two transformants showed wild-type mating ability.
   (a) What is the significance of demonstrating that loss of the plasmid causes loss of both the Leu+ and mating ability+ phenotype simultaneously?
   (b) The isolation of two complementing plasmids helped to localize the STE2 gene within the insert fragments contained in these plasmids. How?
(c) Describe the method used to further localize \textit{STE2} within the 4.3 kbp \textit{Bam}HI fragment shown in Figure 1.

4. Define the term 'allele specific'. Describe the method used to demonstrate that the plasmid-borne complementing gene is not allele specific.

5. Plasmid integration was used to demonstrate that the complementing fragment derives from the \textit{STE2} sequence. Integration occurs via a recombination event between a sequence on the plasmid and a \textbf{homologous} sequence in the host cell's genome. Plasmid pAB503 contains two yeast sequences, \textit{TRPI} and the presumed \textit{STE2}, and therefore it should be able to integrate but \textbf{only} at either of these two positions in the genome. If the insert sequence is not \textit{STE2}, then integration will occur at the genomic site of the homologous sequence and not at \textit{STE2}. Tetrad analysis is used here to demonstrate the site of integration and the results are presented in Table 1.
(a) Using the diagram below, draw the structure of the genomic region that would result from the integration of plasmid pAB503 at \textit{ste2-3}.

(b) Explain why the results for transformants B3-1 and B3-12-3 in Table 1 are consistent with integration at \textit{ste2-3}.
(c) How would these results differ if B3-1 had been crossed to a \textit{MAT}\alpha \textit{ste2-3 TRPI} strain?
(d) How could this plasmid have been targeted to integrate \textbf{only} at the homologous site in the genome?

6. What do the results in Table 2 demonstrate? Why were the results using strains 4277-7, YR-4-3, and 4345-32 included?

7. What is the significance of finding an \textit{\alpha2} protein binding site in the promoter sequence of \textit{STE2}?

8. Describe how a 'hydropathy plot' like the one shown in Figure 4 is calculated. What is the significance of a 'peak' about 20 residues in length with a hydrophobicity of greater than +1?
9. How are the results of the sequence analysis of Ste2 protein consistent with its proposed role as the α-factor receptor? How does Ste2p differ from other known peptide receptors?

10. Ste2p and Ste3p (the α-factor receptor) have similar structures but exhibit no obvious sequence similarity. Nonetheless, the genetic evidence (Article 2, Article 3) suggests that both receptors signal via the same pathway. Discuss.

REFERENCE


ARTICLE 5


1. Describe the phenotype of the M200-6c ste18 mutant strain (MATa sst1 sst2 ste18-1) shown in Figure 1.

2. The authors state, ‘The original ste18 mutant was transformed with a genomic yeast DNA library cloned in the centromere vector YCp50, and colonies were screened for the reappearance of the supersensitivity to α-factor exhibited by the parent strain of the mutant.’
   (a) Considering that 5000–10000 transformants need to be screened, suggest a rapid screening method using replica plating that might be used.
   (b) YCp50 carries the URA3 selectable marker. List the steps needed to clone STE18 from this library in strain MATa sst1 sst2 ste18-1.

3. One-step gene disruption was used to demonstrate that the cloned fragment derives from and contains STE18. What segregation pattern of Ura+ to Ura−, mater to nonmater, would have resulted had the cloned fragment derived from a sequence unlinked to STE18?

4. Discuss the sequence homologies for Ste4p and Ste18p to other proteins in the databases. What sequence motifs of known significance are found in Ste4p and Ste18p?

5. In Table 1, the column labeled ‘STE18’ indicates whether a ste18-LacZ reporter is integrated at STE18.
   (a) Diagram this ‘in-frame’ ste18-LacZ reporter. You need to indicate the approximate position of the junction site between STE18 and LacZ sequences and in what part of STE18 (promoter, ORF, etc.) the junction site is located.
(b) Diagram the protein product produced.
(c) Summarize the results that indicate that STE18 is transcribed in $a$ and $\alpha$ mating type cells but not in $a/\alpha$ diploid cells.

6. Discuss the significance of the finding that strains carrying the null ste4$\Delta$ and ste18$\Delta$ mutations have the same phenotypes as the previously isolated mutations that may be only single amino acid changes.

7. GPA1, STE4, and STE18 appear to encode the components of a heterotrimeric G-protein complex similar to others characterized in mammalian cells. In those systems, in vitro biochemical evidence suggests that the $\alpha$ subunit acts separately from the $\beta\gamma$ subunits and that the disassociated state is the activated state. What genetic results reported in this article are consistent with these findings from the mammalian systems?

8. Summarize the genetic evidence reported in this article that allows the authors to conclude the following. (This is an epistasis analysis and was studied in detail in Chapter 6; see also Articles 6 and 7 of this Case Study.)

(a) Ste4p and Ste18p are both required for activation of the mating type signaling pathway. That is, Ste4p and Ste18p have positive functions in the pathway.
(b) Gpa1p inhibits signaling through the pathway. That is, Gpa1p has a negative function in the mating-type signaling pathway.
(c) The inhibitory action of Gpa1p occurs at an earlier part of the pathway than the function of Ste4p or Ste18p (Table 2).

ARTICLE 6


1. The results reported in Dietzl & Kurjan (1987) and Whiteway et al. (1989) (Article 5) indicate that the STE genes encode components of a switch regulatory pathway as defined in Chapter 6. Discuss the evidence that supports this conclusion.

2. Explain why the anticipated phenotype of constitutive mutations in the mating-type signaling pathway is haploid lethal.

3. Based on your knowledge of the roles of Ste2p, Gpa1p (Sglp1p), and Ste4p, would you expect dominant or recessive constitutive mutations in STE2, GPA1, and STE4? Explain.

4. Describe the red/white colony-sectoring assay. How is it used here to identify clones carrying potential haploid lethal mutations? Include in your answer an
explanation of why candidate haploid lethal mutants would have a non-sectoring phenotype.

5. Describe the procedures undertaken in the analysis of the 87 nonsectoring mutant clones that reduced this number to the final five mutants studied in this article. What phenotypes distinguish haploid lethal mutants affecting the mating-type signaling pathway from other haploid lethal mutants affecting other pathways?

6. For genetic analysis of the haploid lethal mutants, it is essential that cells that have lost the pDJ117 plasmid are capable of mating even though they are incapable of division. Discuss.

7. Describe the experimental procedure used to test dominance of the haploid lethal mutations.

8. Describe the two methods used to demonstrate that the four recessive haploid lethal mutations are alleles of \( SGCI \) (\( GPAI \)).

9. Diagram the cross \( HPL-6 \times HIS3 \). Give the genotype and phenotype (viability and ability to grow in the absence of histidine) of the parents, diploid, and PD, NPD, and TT tetrad spores. (Assume that the mating is between cells of strain 5680-4 (\( his3 \)) and cells of mutant DB6 that have lost the pD117 plasmid.)

10. Table 3 presents the segregation pattern of the mutant phenotype.
   (a) What is the 'mutant phenotype'?
   (b) In the column labeled '3:1', what is the phenotype of the '3' spores?

11. In Table 3, the first two rows demonstrate that \( scgl-4 \) is epistatic to \( ste2-3^{ts} \).
   (a) What data allow the authors to make this conclusion?
   (b) Of 10 tetrads from the W.T. \( \times ste2-3^{ts} \) cross, two give a 3:1 segregation pattern. What does this indicate and how does this help you in your evaluation of the results of the \( scgl-4 \times ste2-3^{ts} \) cross?
   (c) Would you have expected 'cold-sensitive segregants' given the segregation pattern observed? Explain.

12. The results of the cross of \( STE4^{Hp1} \times ste5-3^{ts} \) in Table 3 indicate that \( ste5-3^{ts} \) is epistatic to \( STE4^{Hp1} \).
   (a) Specify these results.
   (b) What is the phenotype (inviability, morphology, mating efficiency) of the \( STE4^{Hp1} ste5-3^{ts} \) double mutant segregants at 34°C and at 22°C?
   (c) Give the genotype and phenotype of the spores of a 3:1 tetrad.
   (d) What is the genotype of the 11 cold-sensitive segregants and is this the expected number given the number of tetrads analyzed? Explain.

13. The authors suggest one possible mechanism for the constitutive activity of the \( Ste4^{Hp1} \) mutant protein. Discuss. Given the same model of the pheromone
response pathway proposed in Figure 2, suggest an alternate mechanism for this Ste4p constitutive mutant. (Assume that some free $\beta\gamma$ is always present.)

REFERENCE

ARTICLE 7

1. Diagram the structure of the $GAL1promoter-STE11\Delta N$ fusion gene. Based on the information given in this article, indicate in your diagram the basepair numbers of the region of $STE11$ included in the fusion, the basepair number of the proposed translation start site, and the predicted size of the protein product (number of residues). How did the authors experimentally demonstrate the expression of a Ste11 protein of the predicted size?

2. Describe the experiments used to demonstrate the following.
   (a) The Ste11$\Delta N$ protein is functional.
   (b) $STE11\Delta N$ is dominant to $STE11$.
   (c) $STE11\Delta N$ is constitutive.

3. Give the specific result(s) from Table 1 that demonstrate the following.
   (a) $STE11\Delta N$ is epistatic to $ste4$.
   (b) ste7 is epistatic to $STE11\Delta N$.
   (c) $FUS3$ and $KSS1$ have overlapping functions in the mating-type signaling pathway.
   (d) $fus3$ and $kssl$ are epistatic to $STE11N$.

4. Ste7 protein exhibits sequence homology with several serine/threonine protein kinases. The authors constructed the $ste7-A220$ mutation to demonstrate that the postulated kinase activity is essential for Ste7p signaling in the mating-type pheromone response pathway. Describe this experiment. Include in your answer the reasons for mutating this specific residue.

5. Discuss the reasons why the authors cannot place the Kss1 and Fus3 kinases at a specific position downstream of Ste11 kinase. Describe a genetic approach (using epistasis analysis) that you might use to determine whether $KSS1$ and $FUS3$ are upstream or downstream of $STE7$. 
ARTICLE 8


Prior to this report, all the mutants isolated based on their resistance to α-factor arrest were also found to be sterile and did not induce FUS1 or other genes regulated by the mating-type pheromone response pathway. In this article the authors search for mutants that separate these phenotypes. That is, they searched for mutants that were resistant to pheromone arrest but were still capable of signaling.

To understand the mechanism of cell cycle arrest by the mating-type pheromones, the reader must become familiar with the regulatory controls of the cell division cycle, particularly those controlling the G1 to S transition. The description in a cell biology text should be sufficient. In Saccharomyces, CDC28 encodes the p34Cdc2 kinase homologue that is the key regulator of both the G1 to S and G2 to M transitions. Cdc28 protein is a cyclin-dependent kinase and is activated by binding with a cyclin protein. Saccharomyces has two classes of cyclins used to regulate the cell cycle encoded by the CLN and CLB genes. CLN1, CLN2, and CLN3 encode the G1 cyclins. That is, they are expressed during the G1 phase, although their expression patterns are distinct. The Cln proteins exhibit sequence homology but are not identical, and binding of any one of these to Cdc28 is sufficient to traverse START. Three different cyclins, encoded by the CLB genes, regulate Cdc28 during the G2 to M transition and are referred to as the G2 cyclins. A culture of unsynchronized cells when treated with pheromone will proceed through S, G2, and M and arrest in G1 at START. In earlier studies Hereford & Hartwell (1974) demonstrated that α-factor arrested cells at START and this was coincident with the requirement for Cdc28 activity. Thus, the G1 cyclins are the likely targets of α-factor arrest. For these reasons, Chang & Herskowitz investigated the CLN genes as targets of FAR1-mediated cell cycle arrest in response to α-factor.

1. Discuss the details of the mutant isolation screen that enabled the authors to isolate this novel class of mutants resistance to α-factor arrest. How are they novel?

2. Describe the following phenotypes of far1 mutants in the presence of α-factor.
   (a) Changes in the cell cycle.
   (b) Colony formation.
   (c) Transcription of downstream target genes like FUS1 and agglutinin.
   (d) Morphological changes.
   (e) Mating competency.

3. What results indicate that Far1p acts only in the α-factor response pathway that regulates cell division and not in other pathways regulating the cell cycle, such as those that respond to changes in nutrient levels?
4. Summarize the evidence that FAR1 expression is regulated by pheromone via the pheromone response pathway.

5. Based on the results shown in Figure 6, the authors conclude that Farlp is a negative regulator of Cln2p and not Cln1p or Cln3p. These conclusions are summarized in Figure 9.
   (a) What evidence indicates that all three Cln proteins are inhibited in MATα cells exposed to α-factor?
   (b) Which mutant allele is epistatic, cln2 or farl? Which gene is downstream?
   (c) What evidence indicates that Farlp is a negative regulator of Cln2p? (Remember that Cln2p is a positive regulator of the G1 to S transition.)
   (d) What evidence indicates that cln1Δ and cln3Δ are not downstream of farl?

6. Discuss the reasons why the authors suggest that the Far1 protein has additional roles in mating other than its role in G1 arrest.

7. Why is it necessary to use the farlΔ null mutation and not a farl point mutation to conclude that Farlp has no role in the mating-type pheromone response pathway itself?

8. Describe how you would select/screen for mutants in the α-factor dependent inhibitor of Cln1p, i.e. the one referred to as X in Figure 9. Be specific with regard to the genotype of the starting strain.

REFERENCE


ARTICLE 9


FUS1 encodes a product required for the fusion of haploid cells during mating. FUS1 expression is induced by exposure to mating type pheromone and induction requires signal transduction via the pheromone response pathway defined by the Ste2, Ste4, Ste18, Ste5, Ste7, Ste11, Fus3/Kss1, and Ste12 proteins. Ste12p, a DNA-binding transcription activator, turns on FUS1 transcription by binding to sites in the promoter. In Article 9 the authors use yet again another approach for the isolation of genes involved in mating-type signal transduction and successfully identify a new STE gene.
1. In this article the authors searched for genes that, when overexpressed, cause the constitutive expression of one of the downstream targets of the mating type pheromone pathway FUS1.
   (a) Diagram the library vector showing the structure of the insertion site of the yeast DNA fragments. At best, only one in six inserts will produce a product normally expressed in yeast. Explain why one in six is the maximum number.
   (b) Diagram the FUS1 reporter construct.
   (c) Outline the steps used to identify 'positive' clones. Start with the selection of transformants (assume the selection marker on the vector is LEU2). Be sure to specify the carbon source at each step.

2. 'Preliminary sequence data suggested that this clone might not contain a full-length gene.' Based on the information in the text, diagram the fusion gene found in the novel clone. Indicate the translation start site of the fusion gene. Based on the sequence of STE20 in Figure 3, what residues are present in the protein product of ste20N?

3. Is overexpressed STE20N dominant or recessive to STE20? What does this suggest with regard to the role of the N-terminal region of Ste20p? Which residues contain the putative kinase domain of Ste20p?

4. Describe the construction of ste20-l and list the complete phenotype.

5. Is Ste20p a positive or negative regulator of the mating-type response pathway? Explain.

6. Describe the expression pattern of STE20.

7. Epistasis analysis was undertaken to place STE20 in the mating type response pathway in relation to the other STE genes.
   (a) When mating efficiency is measured, which is epistatic, STE20N or ste4, ste5, ste11, or ste12? Where does this place STE20 in the pathway: STE4 → STE5 → STE11 → STE7 → STE12
   (b) When growth arrest is measured, which is epistatic, STE20ΔN or ste4, ste5, ste11, or ste12? Where does this place STE20 in the pathway: STE4 → STE5 → STE11 → STE7 → STE12
   (c) Which is epistatic: ste20-ΔI or overexpression of STE4? What phenotype is monitored in this experiment?
   (d) Which is epistatic: ste20-ΔI or overexpression of STE11ΔN? What phenotype is monitored in this experiment?
   (e) Which is epistatic: ste20-ΔI or overexpression of STE12? What phenotype is monitored in this experiment?

8. Based on the conflicting results of their epistasis analysis the authors finally settle on the suggestion that STE20 functions 'prior to STE12' in the mating-type signaling pathway. Discuss the possibility that these results suggest a dual
function for Ste20p in the mating response. One function is a positive one in the mating-type signaling pathway and the second function is in growth arrest.

ARTICLE 10


The results described in Article 9 do not clearly indicate the position of STE20 in the mating type pheromone response pathway. The authors of this article hope to gain insight into this question by isolating multicopy suppressors of an ste20 null mutation.

1. Describe the selection/screen designed by the authors for the isolation of multicopy suppressors of ste20.
   (a) Describe the genotype of the host strain in detail, specifically the ste20 mutant allele used for the search.
   (b) Describe the library. YEp24 carries URA3.
   (c) What phenotype will be used to identify clones carrying a multicopy suppressor?
   (d) Outline the steps in the selection/screen starting with the selection of transformants.

2. List all the phenotypes of ste20-l that are suppressed by plasmid p24-1.

3. Describe how the authors demonstrated that the multicopy suppressor gene in plasmid p24-1 was STE5.

4. Ramer and Davis (1993) (Article 9) reported that overexpression of STE11N did not suppress a ste20 null mutation. Leberer et al. report here that a hyperactive STE11 allele suppresses the mating defect of ste20 mutations. Both articles find that overexpression of STE12 suppresses ste20-1. What type of suppression is this (by-pass, allele specific, or suppression by epistasis)? Where do these results place STE20 in relation to STE11 in the mating-type pheromone response pathway?

5. Compare the structure of the mutant alleles ste20-1 and ste20-2. Use diagrams for your answer.

6. What experimental results suggest that ste20-1 produces a partially functional product? What is the presumed product of ste20-1 (based on your knowledge from Article 9)? Does expression depend on the STE20 promoter? Explain.

7. Summarize the results that indicate the following.
(a) Activation of the mating-type pheromone response pathway by \textit{STE5} overproduction is dependent on the partially functional \textit{ste20-I} allele.
(b) Activation of the mating-type pheromone response pathway by \textit{STE5} overproduction is dependent on \textit{STE4} and \textit{STE18}.

8. What type of suppression is the suppression of \textit{ste20-I} by multicopy \textit{STE5} (bypass, allele specific, or suppression by epistasis)?

9. Choose one of the following two models of the relationship of the \textit{STE4}, \textit{STE5}, and \textit{STE20} genes as best explaining this portion of the mating-type pheromone response pathway. Support your choice using the results presented in this article.

\textbf{Model 1}

\begin{center}
\begin{tikzcd}
\text{STE4} \arrow{d} & \text{STE5} \\
\text{STE20} \arrow{d} & \\
& \text{STE5}
\end{tikzcd}
\end{center}

\textbf{Model 2}

\begin{center}
\begin{tikzcd}
\text{STE4} \arrow{d} & \text{STE5} \\
\text{STE20} \arrow{d} & \\
& 
\end{tikzcd}
\end{center}

10. Describe the structural and functional similarities of Ste5p and Farlp. How was the functional similarity demonstrated?

\textbf{ARTICLE 11}


This article describes the isolation of constitutive \textit{STE5} mutations. Such mutations generate constitutive signaling via the mating-type response pathway and cause cell cycle arrest in haploids. Therefore, investigators isolating such mutations must do so in diploid cells. In previous articles, the \textit{MATa/MAT\textalpha} 'diploid' genotype was reversibly maintained by the introduction of a plasmid-borne copy of the opposite mating-type locus. In this article this is accomplished by a different mechanism.

Chromosome \textit{III} carries the expressed copy of the \textit{MAT} locus and two additional but nonexpressed copies of \textit{MAT}. These so-called silent copies of \textit{MAT} are located near the telomeres of chromosome \textit{III} and are referred to as \textit{HML\textalpha} (left telomere,
copy of $MAT^\alpha$) and $HMR^\alpha$ (right telomere, copy of $MAT^\alpha$). $HML^\alpha$ and $HMR^\alpha$ are not expressed in wild-type cells because of the repressing effects of the $SIR1$, $SIR2$, $SIR3$, and $SIR4$ genes. The products of these genes silence the expression of $HML^\alpha$ and $HMR^\alpha$ via chromosomal position effects (reviewed in Laurenson & Rine, 1992).

A mutation in any one of the $SIR$ genes relieves the repression at the silent $HML^\alpha$ and $HMR^\alpha$ loci. Both loci are expressed and the cell is functionally an $a/\alpha$ diploid. This article uses a temperature-sensitive $sir3^\alpha$ mutation to isolate constitutive $STE5$ mutations. At the permissive temperature Sir3p is functional, the strain expresses only the information at the $MAT$ locus, and the cell is genetically and phenotypically haploid. At the nonpermissive temperature Sir3p is inactive, $MAT$, $HML^\alpha$, and $HMR^\alpha$ are all expressed, and the cell is phenotypically diploid yet genetically haploid.

1. Describe the strategy outlined here for the isolation of haploid-lethal $STE5$ mutations. Why are such mutations expected to be dominant to $STE5$? What is the growth phenotype of $sir3^\alpha$ cells carrying a plasmid-borne $STE5^{\text{Hpl1}}$ mutation at the permissive and nonpermissive temperatures?

2. $STE5$ is a large gene. Moreover, the mutagenesis method introduced multiple mutations. Describe how the phenotypically significant alteration in $STE5^{\text{Hpl1}}$ was localized.

3. Describe the growth phenotype of $STE5^{\text{Hpl2}}$. Is this a generalized effect or specific to a particular phase of the cell cycle? Is this consistent with the expected phenotype of a constitutive $STE5$ mutation?

4. Describe the method used to assay mating efficiency.

5. List the results in Table 2 that indicate that $STE5^{\text{Hpl2}}$ suppresses $ste2::LEU2$. Discuss whether multiple copies of $STE5^{\text{Hpl2}}$ are needed. What form of suppression is this (by-pass, allele specific, suppression by epistasis) and why?

6. Three alleles of $ste4$ are tested in Table 2. Compare the mutational alteration in $ste4-3$, $ste4\Delta::LEU2$, and $ste4::LEU2$. Compare the ability of $STE5^{\text{Hpl2}}$ to suppress each of these alleles. What form of suppression is this (by-pass, allele-specific, suppression by epistasis)? What does this result suggest with regard to the functional relationship between Ste4p and Ste5p?

7. List the results in Table 2 that indicate that $STE5^{\text{Hpl2}}$ does not suppress $ste7::LEU2$, $ste11\Delta::hisG$, or the double $fus3-6::LEU2$ $kss1\Delta::HIS3$. What two interpretations of this result are presented?

8. Based on the results in Tables 3 and 4 discuss the following statement. 'Together, the data indicate that the product of the $STE5^{\text{Hpl1}}$ gene can activate the pheromone pathway in the absence of the pheromone receptor and the $G$ protein but that for full activity it requires $G\beta\gamma$' or overexpression.
Acknowledgments


ARTICLE 12


1. Summarize the selection scheme designed to identify mutations that enhance the phenotype of ste4-ts mutant. Be sure to include the following.
(a) The genotype of the parent strain(s).
(b) Which alleles of ste4 were used? Why did the authors use temperature sensitive alleles and not a ste4Δ mutation?
(c) The growth conditions of the first step in the selection process (carbon source, temperature, etc.).
(d) Potential mutants identified in the selection were screened for their ability to mate at the permissive temperature on galactose plates (step 2). What classes of unwanted mutants would be eliminated by this screen? Explain.
(e) Mutants that passed the screen in ‘c’ were tested further for their ability to mate at the nonpermissive temperature on galactose plates (step 3). What is the purpose of this screen? Explain.

2. Define the term ‘synthetic sterile’.

3. Diagram a cross that would allow the isolation of segregants carrying only the ste-x mutation from a ste4-ts ste-x double mutant isolated by this selection process.

4. Describe the cloning strategy used to isolate ste-x complementing plasmids.

5. Which of the secondary screens described above (step 2 or step 3) should have weeded out the SIR mutations? Why did it fail to do so?

6. The ste18 and ste21 mutations were isolated starting with the parental strain carrying ste4-3510. The ste5 and ste20 mutations were isolated starting with the parental strain carrying ste4-299. Nonetheless, the authors state that ‘the synthetic sterile effects were not allele specific’.
(a) Diagram a cross that would test whether the ste18-14 mutation (isolated in the ste4-3510 strain) is allele specific.
(b) There is no specific information given as to the position of the alterations in ste4-3510 or ste4-299 nor are we informed as to which other ste4 alleles were tested for the authors to conclude that none of the synthetic sterile mutations were allele specific. Discuss why this information would have been valuable.
(c) The original intent of the search for mutations that are synthetic sterile with ste4-ts mutations was to identify proteins that interact directly with Ste4p. Do the results reported here allow the authors to conclude that Ste4p physically interacts with Ste5p? Explain.

7. Discuss a model of enhancement, other than 'allele-specific enhancement', that might explain the results obtained here. That is, mutations in STE18, STE5, and STE20 enhance the phenotype of a ste4-ts mutation. Keep in mind that epistasis analysis of these genes indicates that they could act at the same step.

8. In a recent study Blondel et al. (1999) identify STE21 as MSN5, a member of the nuclear exportin family. They report that Msn5p (Ste21p) is responsible for the pheromone-stimulated export of Far1p from the nucleus. Discuss how a mutation in MSN5 (STE21) could enhance a ste4-ts mutation. Which of the models of enhancement described in Chapter 9 does this represent?

9. Evaluate the results presented regarding the genetic interaction of STE20 and CDC42, particularly in Table 6 and Figures 6 and 7. Of the models presented in Figure 8, which do you think is most consistent with the results presented in this and the other articles of this case study? Explain.

REFERENCE

ARTICLE 13

Epistasis analysis, described in Articles 6 and 7 and elsewhere, places Ste5p upstream of the Ste11, Ste7, and Fus3 or Kss1 kinases in the mating-type pheromone response pathway. The results were consistent with a linear pathway as shown below.

Ste5 protein → Ste11 kinase → Ste7 kinase → Fus3/Kss1 kinase

Nevertheless, evidence was accumulating that this simple pathway was not the full story. Kranz et al. (1994) found that overexpression of Ste5p suppressed point mutations (single residue alterations) in Fus3 kinase and did so in an allele-specific manner. Such a result strongly indicates that Ste5p and Fus3p directly physically interact and is not consistent with the proposed linear pathway that places Ste5p three steps upstream of Fus3 kinase. Kranz et al. (1994) also demonstrated that Ste5p and Fus3p associate with each other even in the absence of a pheromone and even if a catalytically inactive Fus3p mutant is used.
In view of the large size of Ste5 protein and the absence of any recognizable sequence motifs (other than homology to Far1p, another large protein of unknown multiple functions), the authors of this article propose to test the possibility that Ste5p serves as a 'scaffold protein', that is a protein to which other proteins attach in order to come into physical proximity with one another and thereby increase the efficiency of their functional interactions. This article also explores the relationship between Ste20 kinase and Ste5p. Epistasis analysis of Ste20 kinase places it downstream of Ste4p but its relationship to Ste5p remains unclear. Two-hybrid analysis and coimmunoprecipitation are used as complementary approaches to analyze the relationships among these proteins of a MAP kinase signaling cascade.

1. This article uses a 'lexA-based' two-hybrid system. Define the term 'lexA-based'. Include the following in your answer.
   (a) A diagram of the reporter gene.
   (b) A diagram of the structure of the lexA bait construction. Indicate the region encoding the DNA-binding domain, the insertion site for the sequence encoding the bait protein, and the structure of the fusion protein product.
   (c) What is B42?
   (d) A diagram of the structure of the B42 prey construction. Indicate the insertion site for the sequence encoding the prey protein, and the structure of the fusion protein product.
   (e) What is bicoid and what role does it play in this analysis?

2. The results in Table 1 are central to the hypothesis of the authors. That is, Ste5 protein is a scaffold protein capable of interacting with all three of the MAP kinases of the mating-type pheromone response signaling pathway. What evidence in Table 1 supports the following conclusions? Be sure to give the results of the control along with the results of the experiment.
   (a) Ste5p interacts with Ste11p.
   (b) The N-terminal domain of Ste11p is required for the interaction with Ste5p.
   (c) The C-terminal domain of Ste7p is required for the interaction with Ste5p.
   (d) The interaction of Ste5p with Ste11p, Ste7p, or Fus3p is not dependent on the genomic copies of FUS3, STE11, or STE7.
   (e) Ste11p interacts with Fus3p and the interaction is not dependent on Ste5p or Ste7p.

3. An interaction between Ste11p and Ste7p is suggested in Table 1 but does not hold up under detailed analysis.
   (a) Which initial result suggests an interaction between Ste11p and Ste7p?
   (b) Which result indicates that this interaction between Ste11p and Ste7p is indirect and dependent on the genomic copy of STE5 and is mediated by Ste5p?
   (c) Draw a diagram of this interaction.
4. The authors conclude that Ste20p does not interact with Ste5p.
   (a) List the data for both the experiment and the control that support this conclusion.
   (b) Why do you think that the authors do not consider the 33 units or 61 units of activity seen with the Ste5 and Ste11 constructs, respectively, to be significant?
   (c) The authors state, '...LexA-Ste20... functions to repress transcription of a GAL1-LexAop-LacZ gene with a LexA operator between the GAL1 UAS and transcriptional initiation site'. Why is this an important control for this experiment?

5. Describe the results in Figure 1 that demonstrate that Ste11p, Ste7p, and Fus3p interact with distinct regions of Ste5p.

6. Describe the results in Figure 1 that demonstrate that Kss1p and Fus3p interact with the same or an overlapping region of Ste5p.

7. Describe the experiment that indicates that binding of Fus3p to Ste5p is essential for the activation of Fus3 kinase.

8. Discuss the functional significance of the finding that Ste5p binds to the N-terminal domain of Ste11 kinase.

REFERENCE

ARTICLE 14

1. Discuss the model of the ste4 haploid-lethal mutant selection depicted in Figure 1A. List the following.
   (a) The genotype of the strain used for the selection. (Include the plasmid genes.)
   (b) The growth conditions used for the identification of the clones carrying haploid-lethal mutations.
   (c) The STE4 mutagenesis method.

2. Discuss the significance of obtaining mutations only in the region of Ste4p between residues 126 and 150.
3. Describe the method used to isolate suppressors of the ste4 haploid-lethal mutations. Be specific about the ste4 mutant allele used for this selection.

4. List the results that indicate that GPAI-E307K is an allele-specific suppressor of the STE4 haploid-lethal mutations. Draw a model of the interaction of Gpa1p and Ste4p based on these results. Indicate the location (within the region of interaction or not) of the various mutant alterations in Gpa1p and Ste4p identified in this study.

5. The results in Figure 2 and Table 1 indicate that the GPAI mutation Hls 4.3 is a silent mutation. That is, it exhibits wild-type-like activity.
   (a) Discuss the results that support this conclusion.
   (b) What results would you have expected if the GPAI mutation Hls 4.3 had interfered with the activity of Gpa1p?

6. Table 2 uses two-hybrid analysis to explore the direct interaction of the GPAI Hls 4.3 mutant protein with wild-type Ste4p protein and with the Hpl 21.3 mutant protein.
   (a) Which results indicate that the interaction between the Gpa1 Hls 4.3 mutant protein and Ste4p or Ste4 Hpl 21.3 mutant protein is comparable? How is this consistent with the phenotype analysis reported in Figure 2 and Table 1?
   (b) Which results indicate that the Ste4 Hpl 21.3 mutant protein interferes with the interaction with Gpa1 protein?
   (c) Which results indicate that the GPAI mutation Hls 4.3 re-establishes the interaction with Ste4 Hpl 21.3 mutant protein?
   (d) Which results indicate that the interaction between these mutant proteins is allele specific?

7. Discuss other Gpa1 mutations in the region of residue 307 that strengthen the hypothesis that this portion of the Gα subunit is involved in the interaction with Gβγ and may play an important role in the selectivity of the interaction of different Gα subunits with their specific Gβγ targets.

ARTICLE 15


Chenevert et al. (1994) devised a screen to identify mutants defective in the ability to undergo the properly oriented morphological changes (schmoo formation) associated with pheromone exposure. They reasoned that such mutants would be capable of mating to a wild-type strain but should exhibit defects when paired with an enfeebled mating partner. Among the several mutant genes identified, Chenevert et al. (1994) identified new alleles of FAR1 that they called far1s alleles. Article 15 characterizes these far1s alleles.
Chang & Herskowitz (Article 8) identified FAR1 and characterized its role in cell cycle arrest. They demonstrated that Farlp functions as a negative regulator of the G1 cyclin Cln2p. This inhibitory effect involves the direct binding of Farlp to Cdc28p/Cln2p cyclin-dependent kinase but, in contrast to other cyclin kinase inhibitors, Farlp binding does not appear to result in the inhibition of kinase activity and thus the mechanism of action is novel (Peter & Herkowitz, 1994; Gartner et al., 1998). Article 15 focuses on quite a different function of Farlp, namely cell polarization during schmoo formation.

Chang & Herskowitz (Article 8) suggested a role for Farlp in cell polarization during mating based on their initial analysis of different far1 mutant phenotypes. Additionally, Chang (1991) describes farl-c, a C-terminal truncation mutation, capable of cell cycle arrest in response to pheromone but defective in mating, perhaps due to an inability to orient toward the mating partner. These studies illustrate the value of detailed analysis of the pleiotropic phenotypes of multiple mutant alleles.

1. Describe the pheromone confusion assay.
2. Describe the orientation assay.
3. Describe in detail the pleiotropic phenotypes of far1-s alleles. Compare the phenotype of strains carrying the four different far1-s alleles described in Article 15 with wild-type FAR1 strains and with the phenotype of strains carrying other far1 mutant alleles. Where possible, include each of the phenotypes listed below.
   (a) Cell cycle arrest.
   (b) Mating defects with different partners.
   (c) FUS1 transcription.
   (d) Far1 protein expression.
   (e) Mating confusion.
   (f) Orientation to pheromone gradient.
4. Describe the experiments that demonstrate that Farlp has a function in mating in addition to its role in cell cycle arrest.
5. Describe the experiment and the analysis of the experimental results that allows the authors to conclude that the far1-s mutants orient towards the incipient bud site. What does this suggest with regard to the structure of the incipient bud site while the cell undergoes reorientation for schmoo formation?
6. Four far1-s alleles were isolated, sequenced, and characterized. Discuss the value of analyzing multiple alleles. How might their conclusions differ if mutant B4 were not available?
7. farl-60F3 complements farls-D1. Assuming that the Far1 protein does not form homomultimers, propose a mechanism for this intragenic complementation.
REFERENCES


ARTICLE 16


In addition to identifying far1-s mutants, Chenevert et al. (1994) isolated the mutant alleles of several other genes involved in polarized morphogenesis during mating (schmoo formation) as well as in vegetative cell division. These included alterations in CDC24, a GDP-GTP exchange factor for Cdc42p, referred to as cdc24-m mutants because they affect mating at permissive temperatures in addition to having a defect in cell division at higher temperatures. Cdc42p is a small GTPase in the same family of proteins as the mammalian Rho1p and is involved in organizing the actin cytoskeleton (reviewed in Johnson, 1999; Pruyne & Bretscher, 2000; Takai et al., 2001).

Chenevert et al. (1994) also identified schmooless alleles of BEM1, a gene previously shown to be involved in cell polarization during vegetative budding (Chant et al., 1991; Chenevert et al., 1992). Reports that Bem1 protein binds to actin and interacts with Cdc42p, Cdc24p, Farlp, Ste5p, and Ste4p suggested the possibility that these proteins form a large complex that is essential for directing the recruitment of the actin cytoskeleton to the region of the cell surface exposed to the highest concentration of pheromone (Peterson et al., 1994; Leeuw et al., 1995; Lyons et al., 1996; Park et al., 1997; reviewed in Pruyne & Bretscher, 2000). The authors of Article 16 used two-hybrid analysis to demonstrate this proposed complex and to explore specific interactions among the components of the complex.

1. Diagram the FAR1 ‘bait’ construction, the BEM1 ‘prey’ construction, and the reporter gene used to demonstrate an interaction between Farlp and Bemlp.

2. What experimental data supports the statement, ‘Farlp preferentially bound to Cdc42p in its active GTP-bound state, . . .’?

3. What experimental data are presented to support the specificity of the Farlp–Cdc42p interaction?
4. What experimental data indicate that the Farlp interaction with Cdc42p is not direct but is mediated via Bemlp?

5. What pairs of constructs would you test to explore the interactions of Ste20p with the components of this complex? Be sure to examine the possibility that the interactions you detect are not direct and propose how you might test this.

6. Comment on the following. Interactions between two heterologous (nonyeast) proteins detected using the yeast two-hybrid system are most probably direct.

7. Figure 2 shows the two-hybrid results obtained with the far1-s mutant alleles B4 and H7. Discuss how these results are consistent with the phenotype exhibited by strains containing these mutations.

8. The authors cite unpublished results describing STE4 mutant alleles that cause defects in mating but not pheromone signaling. Based on the results described in this article, propose a possible mechanism for this class of ste4 mutation. Outline a series of experiments including two-hybrid analysis and at least one other genetic approach that you might use to investigate this novel class of STE4 alleles to support your hypothesis.

REFERENCES


This article uses the genome-wide transposon mutagenesis approach to identify additional genes involved in cellular processes involved in mating such as agglutination, polarized growth for the formation of the mating projection, cell fusion, and nuclear fusion. Searches for mutants exhibiting defects in mating have identified a number of genes involved in these processes. However, it is likely that many genes have been missed possibly because of redundancy in the *Saccharomyces* genome or because alterations in these genes do not cause defects severe enough to produce a sufficiently distinct phenotype. The approach described here uses transposon mutagenesis (see Chapter 12) to produce random *lacZ* fusions to ORFs throughout the genome and to screen these for pheromone regulation.

1. Describe the *lacZ* insertional mutagenesis scheme used in this article to randomly tag genes in the *Saccharomyces* genome. Include:
   (a) The structure of a typical library plasmid carrying a yeast DNA fragment with a Tn insert creating a *lacZ* fusion.
   (b) The genotype of the host strain into which the library was transformed.
   (c) How were transformants selected?
   (d) How were transformants screened to identify fusions to pheromone-regulated genes?

2. For this study:
   (a) Why did the authors use a *bar1Δ* strain?
   (b) Why were both haploid *MATa* and homozygous *MATa/MATa* diploid strains used?

3. List the phenotypes used to test the novel pheromone-regulated genes identified in this study for a potential affect on mating. Describe the reasons for selecting *FIG1*, *FIG2*, *FIG3*, and *FIG4* for further study.

4. What is a PRE sequence and what is the significance of finding PRE sequences in the promoter regions of *FIG1*, *FIG2*, *FIG3*, and *FIG4*?

5. Describe the method used to demonstrate that cell cycle arrest does not alter the expression of the *FIG* genes.

6. Describe the complete phenotype of the *fig2* mutant in detail. Include results discussed throughout this article.

7. Describe the Fig2 protein. Include sequence motifs and the results of cellular localization studies. What do the authors propose is a likely function of Fig2p?
8. The study identified 54 pheromone-regulated genes but the authors estimate that there are between 67 and 132 such genes in the *Saccharomyces* genome. Describe how they derived this estimate.

9. Only nine previously known pheromone-regulated genes were identified in this screen.
   (a) Name one gene that you know to be pheromone regulated that was not identified.
   (b) How do the authors explain the fact that they missed many genes?
   (c) How might the Tn library be improved so as to make the mutagenesis method more random?