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# —•—— XIII Yeast Mapping Reports

# Localization of the *FAR3* Gene: Genetic Mapping and Molecular Cloning Using a Chromosome Walk-'n'-Roll Strategy

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*FAR3* is a newly-discovered yeast gene required specifically for pheromone-mediated cell cycle arrest. I have used strains harboring the *far3-1* mutation to map the gene to the right arm of chromosome XIII, establishing the gene order *CEN13-LYS7-MCM1-FAR3*. I cloned the *FAR3* gene based on its genetic map position using a strategy that combined chromosome walking and a related technique termed 'chromosome rolling'. In addition to the genetic and physical localization of *FAR3*, I present data that suggest corrections to the tentative map positions of *VAN1* and *ARG80*.

KEY WORDS — *Saccharomyces cerevisiae*; chromosome XIII; *FAR3*; *MCM1*; *LYS7*; *VAN1*; *ARG80*

## INTRODUCTION

As a prelude to conjugation, haploid yeast participate in cell–cell communication by secreting and responding to mating pheromones. Binding of pheromone to its cognate receptor activates a signal transduction pathway that leads to cell cycle arrest and transcription induction of a small set of genes (for a review, see Sprague and Thorner, 1992). In an effort to better understand the mechanism of pheromone-mediated cell division control, I have isolated mutants that are defective in cell cycle arrest and not in signal transduction *per se* (details to be presented elsewhere). One class of recessive mutants has identified a new gene, *FAR3* (mating factor arrest). Here I report the genetic and physical localization of *FAR3*. I also present data that correct the tentative map positions of *VAN1* and *ARG80*.

## RESULTS

### *Genetic mapping of* FAR3

Haploid yeast carrying the *far3-1* mutation are defective in pheromone-mediated cell cycle arrest (data not shown). To determine whether *FAR3* was distinct from the many genes known to be involved in either pheromone response or cell cycle control, I mapped the gene. Initial crosses demonstrated that *FAR3* was linked to its centromere, and I took advantage of this linkage to design a strategy for assigning *FAR3* to a particular chromosome. Since *FAR3* demonstrated centromere linkage relative to *TRP1*, it was expected to show direct linkage to a centromere-linked marker on one of the remaining 15 chromosomes. Tetrad analysis was performed on *MAT***a**/*MAT*α diploids heterozygous at *FAR3* and at least one other centromere-linked marker (Table 1). Diploids heterozygous for six of the centromere-linked markers were constructed by mating a *far3-1* haploid to various haploid strains that already existed in the laboratory collection. Tetrad analysis on these diploids tested linkage of *FAR3* to the centromeres of chromosomes II, III, IV, VII, VIII and IX (Table 1). To create suitable diploids for analysing linkage to markers on the remaining chromosomes, I transformed a *MAT***a**/*MAT*α *far3-1*/+ heterozygote with a series of integrating DNAs representing known centromere-linked markers. In lieu of confirming each integration event by Southern blot analysis, I analysed two independent

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Table 1. Mapping data for *FAR3* and centromere-linked markers.

| Chromosome | Marker | Tetrads scored | Ascus type D1 | D2 | T | PD | NPD | T |
|---|---|---|---|---|---|---|---|---|
| I | *ade1* | 16 | 4 | 5 | 7 | | | |
| II | *fus3* | 27 | | | | 7 | 5 | 15 |
| III | *leu2* | 12 | 2 | 7 | 3 | | | |
| III | *MAT* | 90 | | | | 25 | 20 | 45 |
| IV | *trp1* | 365 | | | | 99 | 88 | 178 |
| V | *ura3* | 14 | 2 | 6 | 6 | | | |
| VI | *sec4* | 14 | 3 | 3 | 8 | | | |
| VII | *kss1* | 13 | | | | 4 | 2 | 7 |
| VIII | *arg4* | 67 | | | | 8 | 21 | 38 |
| IX | *bar1* | 36 | | | | 7 | 10 | 19 |
| X | *met3* | 8 | 2 | 2 | 4 | | | |
| XI | *ren1* | 23 | 6 | 8 | 9 | | | |
| XII | *ppr1* | 26 | 7 | 4 | 15 | | | |
| XIII | *mcm1* | 8 | 8 | 0 | 0 | | | |
| XIII | *rad52* | 15 | 0 | 4 | 11 | | | |
| XIV | *cen14* | 20 | 4 | 5 | 11 | | | |
| XV | *whi2* | 26 | 4 | 3 | 19 | | | |
| XVI | *swi1* | 46 | 7 | 11 | 28 | | | |

D1, ditype with configuration [*far3-1*, 'marked' genetic marker]; D2, ditype with configuration [*FAR3*, 'marked' genetic marker]; T, tetratype; PD, parental ditype; NPD, non-parental ditype.

Table 2. Mapping data for *FAR3, MCM1, LYS7* and *VAN1*.

| Strain | Interval | Ascus type PD | NPD | T | Segregation FD | SD | Map distance (cM)* |
|---|---|---|---|---|---|---|---|
| SY2520 | *far3-mcm1* | 76 | 0 | 7 | | | 4·2[a] |
| | *far3-trp1* | | | | 29 | 54 | 32·5[b] |
| | *mcm1-trp1* | | | | 36 | 47 | 28·3[b] |
| JY2 | *mcm1-lys7* | 69 | 0 | 7 | | | 4·6[a] |
| | *mcm1-met3* | | | | 37 | 36 | 24·7[b] |
| | *lys7-met3* | | | | 43 | 30 | 20·5[b] |
| | | D1 | D2 | T | | | |
| SY2691 | *far3-van1* | 5 | 9 | 29 | | | Unlinked† |
| | *far3-trp1* | | | | 22 | 21 | 24·4[b] |
| | *van1-trp1* | | | | 16 | 27 | >25·0[b] |

*Standard formulae were used to calculate [a]direct and [b]centromere linkage (Sherman and Wakem, 1991).
†See text.
Abbreviations are the same as those defined in the footnote of Table 1.

transformants for each marker (the data from one independent transformant for each marker DNA is presented in Table 1). In each case the transformed marker sorted 2 : 2, indicating that the DNA had integrated at a unique site. Since the transforming DNAs were free to recombine with

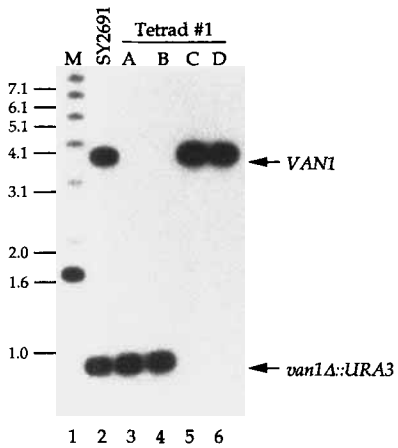


Figure 1. Southern blot confirmation of the *van1Δ::URA3* gene replacement in SY2691. *Hin*dIII-digested genomic DNA was prepared from the a/α diploid SY2691 (lane 2) and from the four meiotic products of a tetrad analysed from SY2691 (lanes 3–6). The probe was a radiolabeled 0·8 kb *Eco*RI/*Pst*I fragment from pNV14 (Kanik-Ennulat and Neff, 1990). Lane 1, 1 kb ladder DNA marker (Bethesda Research Laboratories).
Note added in proof: the *VAN1* DNA probe used in Figure 1 hybridizes with ATCC prime lambda clonee 70665 (WashV #6041). Thus, *VAN1* physically maps between *PHO84* and *FUN81*, near the telomere on the left arm of chromosome XIII.

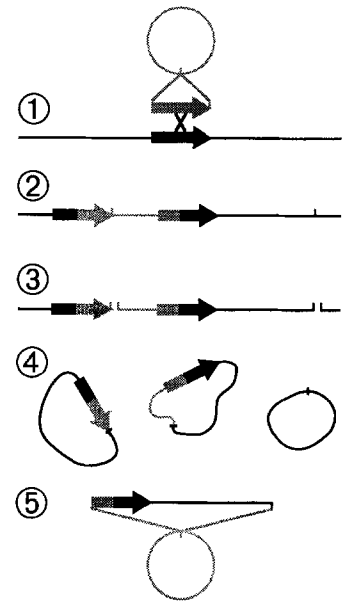


Figure 2. Outline of the chromosome rolling strategy. (1) Subclone DNA marker into a suitable vector (e.g., pRS306; Sikorski and Hieter, 1989), then integrate it into the homologous chromosomal region in yeast. (2) Use Southern blot analysis to confirm integration and to identify a restriction enzyme that cuts at one end of the intervening vector sequence and at a distal site in the chromosome. (3) Digest genomic DNA. (4) 'Roll' the chromosome fragments into circles by unimolecular ligation. (5) Transform *E. coli*, selecting for vector DNA; characterize the clone and repeat the whole process if necessary.

either parental homolog of the diploid, the ditype tetrads were grouped into two classes designated ditype 1 (D1) and ditype 2 (D2) (Table 1). Direct linkage of *FAR3* to a particular centromere-linked marker would be revealed by a significant deviation from equality of the number of D1 and D2 tetrads recovered. By this measure, *FAR3* showed direct linkage to both *MCM1* and *RAD52*, which are located on the right and left arms, respectively, of chromosome XIII (Mortimer *et al.*, 1992). Direct linkage to both *MCM1* and *RAD52* assigned *FAR3* to chromosome XIII.

The data presented in Table 1 indicated that *FAR3* is tightly linked to *MCM1* on chromosome XIII. To establish the position of *FAR3* relative to *MCM1* and *CEN13*, I analysed tetrads from strain SY2520 (relevant genotype *MAT***a**/*MATα far3-1*/+ *MCM1::pRS306::MCM1*/+ *trp1*/+). SY2520 had been created in the transformation strategy described above, and the integration of *MCM1* DNA was subsequently confirmed by Southern blot analysis (data not shown). Among the ditype tetrads (76 out of 83 total tetrads), *far3-1* and *MCM1::pRS306::MCM1* were always coupled; I inferred that this ditype class (D1) was parental. In the seven tetratype tetrads recovered for *FAR3* and *MCM1*, *MCM1* showed first-division segregation relative to *TRP1*. Thus, *FAR3* is located 4·2 cM centromere distal to *MCM1*.

The positioning of *MCM1* on the current genetic map was derived from both genetic and physical data. *MCM1* was genetically mapped near *LYS7* based on a small data set obtained from a two-point cross (Passmore *et al.*, 1988). *MCM1* was then placed centromere distal to *LYS7* because *MCM1* (also known as *FUN80*) is physically adjacent to *ARG80*, a gene that had been previously mapped centromere distal to *LYS7* (Dubois *et al.*, 1987; Mortimer *et al.*, 1992; Mortimer and Schild, 1980; Passmore *et al.*, 1988). Because the strategy for the molecular cloning of *FAR3* relied on its genetic map position (see below), I tested the correctness of the map order by a three-point cross. In this cross (JY2, relevant genotype *MCM1::pRS306::MCM1*/+ *lys7*/+ *met3*/+) the relative order of *LYS7* and *MCM1* with respect to *CEN13* is based on segregation with *MET3*, which is near *CEN10* (Mortimer

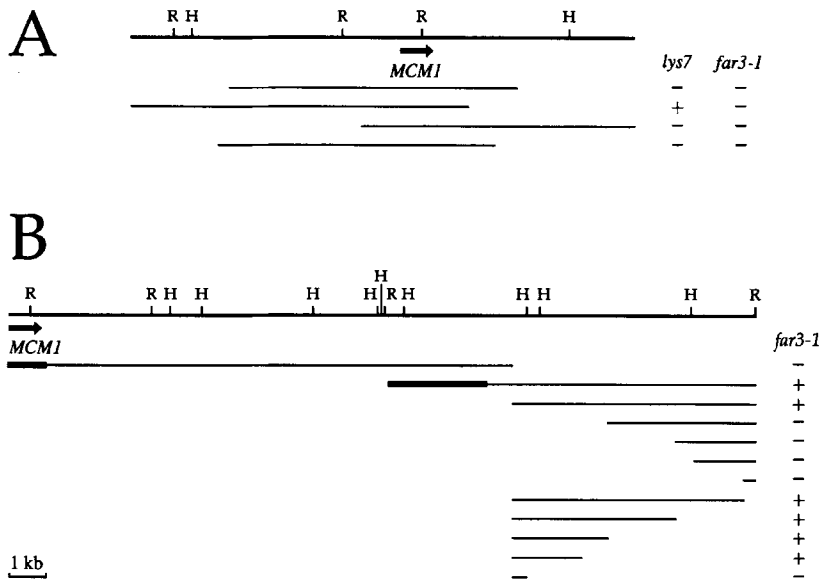


Figure 3. Molecular cloning of *FAR3* by chromosome walking-'n'-rolling. An *Eco*RI (R) and *Hin*dIII (H) restriction map is drawn for the DNA spanned by the chromosome walking (A) and chromosome rolling (B) experiments. The position and polarity of *MCM1* is indicated by an arrow below each restriction map. Lines below the restriction maps represent DNA segments corresponding to the four overlapping *MCM1* clones (A), the chromosome roll products (B, top two lines), and the deletion series of the second chromosome roll product (B, lower ten lines). The ability of each DNA segment to complement the *lys7* and *far3-1* mutations is indicated. The bold regions on the chromosome roll products represent the regions used for targeting integrative transformation.

*et al.*, 1992). The data presented in Table 2 support the published map order, and taken together with the *FAR3* data presented above establish the gene order of *CEN13-LYS7-MCM1-FAR3*.

*VAN1* has also been positioned approximately 8 cM centromere distal from *LYS7* (Mortimer *et al.*, 1992), implying that it is near *FAR3*. The *VAN1* meiotic mapping data were inconsistent: *VAN1* showed tight linkage to *LYS7*, yet appeared unlinked to both *RAD52* and *CEN13* (Kanik-Ennulat and Neff, 1990; Mortimer *et al.*, 1992). To test for possible linkage between *VAN1* and *FAR3*, I analysed tetrads from a diploid strain that had been transformed with *van1Δ::URA3* DNA (pNV14; Kanik-Ennulat and Neff, 1990). Southern blot analysis (Figure 1) confirmed the gene replacement in strain SY2691 (relevant genotype *MAT***a**/*MAT*α *far3-1*/+ *van1Δ::URA3*/+ *trp1*/+ *ura3*/*ura3*). Again, since the transformed DNA was free to recombine with either parental homolog of the diploid, the ditype tetrads obtained from SY2691 were grouped into two classes designated ditype 1 (D1) and ditype 2 (D2). I obtained both D1 and D2 class tetrads for *FAR3* relative to *VAN1* (Table 2); the numbers of tetrads comprising the two ditype classes were not significantly different from each other at the 5% level, indicating that *VAN1* is unlinked to *FAR3*. Furthermore, *VAN1* does not lie between *CEN13* and *FAR3*, based on the segregation of *FAR3* and *VAN1* with *TRP1*. Hence, *VAN1* is unlinked to the *CEN13-LYS7-MCM1-FAR3* region and is probably unlinked to *CEN13* on the left arm. It should be noted, however, that *VAN1* (and an adjacent gene, *ATR1*) physically maps to chromosome XIII by chromosome blot hybridization experiments (Kanazawa *et al.*, 1988; Kanik-Ennulat and Neff, 1990).

The genetic data presented above are summarized in Figure 4A. The distance between *CEN13* and *FAR3* was derived from the segregation data presented in Table 1 for *FAR3* relative to *TRP1*. The calculated distance of 24·4 cM is probably an overestimate of the actual genetic distance for two reasons. First, the calculation ignored the genetic distance between *TRP1* and *CEN4*, which is

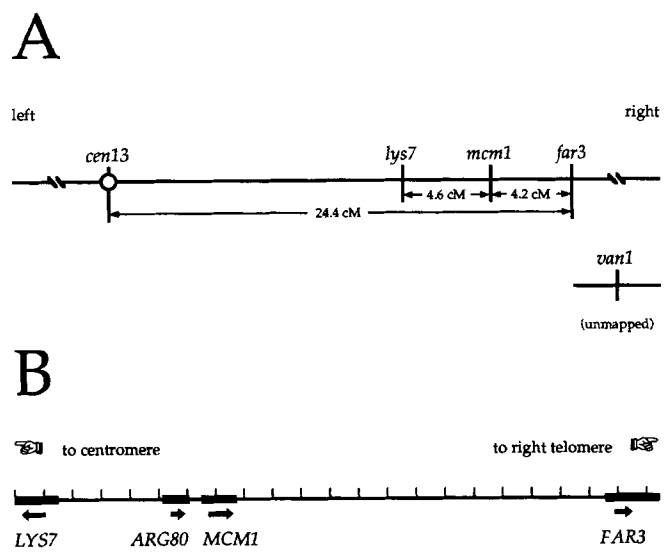


Figure 4. Genetic (A) and physical (B) maps of the *CEN13-LYS7-MCM1-FAR3* region on chromosome XIII. Ticks in the physical map mark 1-kb intervals. The thick bars and arrows represent sequenced regions and open reading frames, respectively, for *LYS7* (J. Horecka and P. Kinsey, unpublished data), *ARG80* (Dubois *et al.*, 1987), *MCM1* (Dubois *et al.*, 1987; Passmore *et al.*, 1988) and *FAR3* (J. Horecka, unpublished data).

approximately 1·1 cM in this strain background (Horecka and Sprague, 1994). Second, although the *TRP1-FAR3* segregation data compiled in Table 1 was obtained from congenic yeast strains, the data from the diploid heterozygous for the *MCM1::pRS306::MCM1* allele (SY2520, a subset of the data in Table 1 and presented separately in Table 2) yields an unusually large *CEN13-FAR3* genetic distance compared to the other diploids (32·5 vs 22·0 cM, $\alpha<0{\cdot}05$).

### *Molecular cloning of* FAR3 *by chromosome walking-'n'-rolling*

I was unable to clone the *FAR3* gene by simply transforming a *far3-1* strain with a genomic library and complementing the mutant phenotype (data not shown). Therefore, I used a strategy that took advantage of the genetic map position of *FAR3*. For chromosome XIII, physical distance is related to genetic distance by approximately 3 kb/cM (Mortimer *et al.*, 1992), suggesting that *FAR3* and *MCM1* are separated by approximately 13 kb. I therefore elected to clone *FAR3* by chromosome walking, starting from the *MCM1* gene. I obtained a set of four overlapping *MCM1* clones by transforming a genomic library into a yeast strain harboring a conditional *mcm1* mutation (data not shown). Each *MCM1*-containing clone was transformed into both *far3-1* and *lys7* strains to test for complementation. A restriction map of the *MCM1* region, an alignment of the inserts from the overlapping library clones, and the ability of the library clones to complement the *far3-1* and *lys7* mutations are presented in Figure 3A. I recovered one *MCM1* clone that complemented *lys7*, but none of the clones extended far enough from *MCM1* to complement *far3-1*.

To obtain more DNA on the inferred *FAR3*-side of *MCM1* I used the allele rescue technique (Rothestein, 1991; Stiles *et al.*, 1981) in a reiterative fashion, enabling me to carry out a unidirectional 'chromosome roll' from a precise physical location toward the genetically-defined *FAR3* locus. The general procedure for chromosome rolling is outlined in Figure 2. To initiate chromosome rolling toward the *FAR3* locus I used a tetratype segregant from SY2520 that has the configuration *MCM1::pRS306::MCM1-FAR3* (pRS306 is a *URA3*-marked yeast-*E. coli* shuttle vector; Sikorski and Hieter, 1989). In this initial attempt I recovered a 13·5 kb segment of chromosomal DNA on the *FAR3*-side of *MCM1*,

but unfortunately it failed to complement the *far3-1* mutation. The procedure was therefore repeated by subcloning a small segment of DNA from the *MCM1*-distal end of the first roll product into pRS306 and using that segment to target integration. On this second attempt I recovered a 10 kb segment of chromosomal DNA that complemented the *far3-1* mutation (Figure 3B). This 10 kb DNA segment was subjected to a systematic deletion analysis that narrowed the *far3-1*-complementing activity to a 1·8 kb restriction fragment (Figure 3B). Subsequent experiments demonstrated that this 1·8 kb restriction fragment contains the *FAR3* gene (data not shown).

The combined data from the chromosome walking and chromosome rolling experiments are presented in Figure 4B as a physical map that spans approximately 22 kb from *LYS7* to *FAR3*. Included in this map is *ARG80*, a gene that resides upstream of the *MCM1* coding region (Dubois *et al.*, 1987; Passmore *et al.*, 1988). Restriction map analysis indicates the gene order *LYS7-ARG80-MCM1* (data not shown); this order corrects the order of these three genes on the current genetic map (Mortimer and Schild, 1985).

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