Genetic Control of Yeast Mannan Structure

MAPPING THE FIRST GENE CONCERNED WITH MANNAN BIOSYNTHESIS*

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SUMMARY

Two “wild type” strains of the yeast Saccharomyces cerevisiae, which differ in the structure of their cell wall mannan, have been hybridized and the genetic control of the mannan structure expressed in the diploid has been investigated. The mannan of one strain (X2180) is characterized by the presence of a mannotetraose side chain, while the other (strain 4484-24D) possesses a mannosylphosphorylmannoside side chain in place of the mannotetraose unit. The diploid had the X2180 mannan chemotype rather than an average mixture of the two.

Sporulation and tetrad analysis of the diploid hybrids demonstrated that the two mannan chemotypes segregated 2:2 as though the difference were under the control of a single gene, and from a detailed genetic analysis we have mapped the “dominant” gene to chromosome V, tightly linked to ura3 and the centromere. It is the first gene concerned with mannan biosynthesis to be placed on the yeast genetic map and it has been designated mnn1. From the observed differences between the structures of the mannans of strains X2180 and 4484-24D, we conclude that the gene is involved in the synthesis of an α-1→3-mannosyltransferase which adds the terminal α-1→3-linked mannotriose unit to α-1→2-linked mannotriose side chains in the mannan. The mechanism which prevents the expression of the mannosylphosphate group on the surface of the hybrid diploid cell is unknown, but it must be related to the processes which control the organization of the mannan in the cell wall.

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transferase, which, when present, modifies the expression of a
mannosylphosphate transferase. The implications of this
finding with respect to the mechanism of mannan biosynthesis
are discussed.

EXPERIMENTAL PROCEDURE

*S. cerevisiae* X2180-1A (a mating type), *S. cerevisiae* 4484-24D
(α mating type), and the diploid from a cross between these two
strains were used in this study. The yeasts were grown and
mannan was isolated by the general procedure of Thieme and
Ballou (5). Carbohydrate was measured by the phenol-sulfuric
acid assay (8), and phosphate according to Bartlett (9). Im-
munochemical analysis (6), acetolysis (10), and methylation
analysis and characterization by mass spectrometry (11) fol-
lowed published procedures.

Antisera were prepared by injecting rabbits with heat-killed
whole cells (2). Antiserum was made specific for the tetra-
saccharide determinant of X2180 mannan by adsorbing the
serum against X2180 cells with heat-killed 4484-24D cells.
Antiserum specific for the α-α-mannosylphosphodiester side
chain was made in a similar manner by adsorbing serum against
4484-24D cells with X2180 cells. Agglutinations were done

RESULTS

Chemical Characterization of 4484-24D Mannan—Acetolysis of
the 4484-24D mannan yielded the neutral fragments mannose,
mannobiose, and mannotriose in the approximate ratios 1:2:1
(Fig. 2C). The peaks from right to left are mannose (M1), mannobiose (M2), mannotriose (M3), and mannotetraose (M4).

The rationale for genetic mapping, along with the procedures
and media for hybridization, sporulation, ascus dissection,
soring mating type or nutritional markers have been described
(12–14). The sporulated cultures were treated with “Glusulase”
(Endo Laboratories) to remove the ascus wall and four-spored
asci were separated by micromanipulation.

Fig. 1. Detailed structures illustrating the polysaccharide
component of the mannan from (A) *Saccharomyces cerevisiae*
X2180 and S288C, and (B) *Kloeckera brevis* and *S. cerevisiae* 4484-
24D. The molar ratios of the different side chains are not
indicated, and it is not known whether the side chains occur in a defi-
nite order or are arranged randomly.

Fig. 2. Acetolysis patterns of the mannans from (A) X2180-1A,
(B) the hybrid cross of X2180-1A × 4484-24D, and (C) 4484-24D.
The peaks from right to left are mannose (M1), mannobiose (M2),
mannotriose (M3), mannotetraose (M4), and mannotriose phos-
phate (M4P). The solid line is carbohydrate and the dashed line
is total phosphate. A column (2 × 200 cm) of Bio-Gel P-2 (200 to
400 mesh) was used and was eluted with water.
patterns (Fig. 2) which show that the diploid cross gave the phosphate side chains in the latter. While X2180-1A did have 44%24D, a result consistent with the presence of the mannosyl-phosphate side chains in its mannan, the amount was much lower than that in the 4484-24D strain. Agglutination reactions with specific antisera supported the conclusion that the surface antigens of the diploid cross were very similar to those of the X2180-IA strain, and unlike those of strain 4484-24D (Table I), also segregated for two heteroeygous centromere-linked marker genes that collectively identified 14 linkage groups in the Saccharomyces genome (Table II). Mannan of the spore clones resembled the X2180-IA mannan. The precipitin curves (Fig. 3) established that both mannans possessed the mannotetraose side chain determinant, and that neither had a significant amount of the mannosylphosphate side chain. On the other hand, the strong cross-reaction of 4484-24D mannan with anti-K. brevis serum, which was specific for the mannosylphosphate side chain, confirmed the presence of this group in the 4484-24D mannan.

**Immunochemical properties of the isolated mannans showed that the polysaccharide from the hybrid diploid closely resembled the X2180-1A mannan.** The precipitin curves (Fig. 3) confirmed the presence of this group in the 4484-24D mannan. The strong cross-reaction of 4484-24D mannan with anti-K. brevis serum, which was specific for the mannosylphosphate side chain, confirmed the presence of this group in the 4484-24D mannan.

**Genetic Studies—Originally detected in a doubly disomic strain (4484-24D, isolated by S. Fogel), the phosphomannan structure was found to segregate as a simple, recessive, Mendelian entity in 10 diverse hybrids analyzed.** The diploid cells in each hybrid possessed a mannan profile indistinguishable from the wild type standard cultures X2180 or S288C (Fig. 3). A total of 197 fully viable tetrads was recovered from the 10 hybrids. Among these, the meiotic distribution of the mannotetraose marker was monitored relative to 22 biochemical marker genes that collectively identified 14 linkage groups in the Saccharomyces genome (Table II). Mannan of the spore clones was scored by agglutinability of the whole cells with antiserum specific for the tetrasaccharide side chain. Of the 197 tetrads analyzed, 194 exhibited a 2+:2- segregation pattern for tetrasaccharide against no tetrasaccharide, a pattern consistent with Mendelian expectations for a single gene difference. Gene conversions (3+:1- variety) were observed in three tetrads. In tetrads which were also tested with antiserum specific for the phosphodiester side chain, a reciprocal 2+:2- segregation was seen for the mutant locus carried in a trans relationship to the wild type allele specifying the tetrasaccharide. Among tetrads doubly scored in the manner described above, and even including the convertant tetrads, no recombination between these two characteristics was observed.

**Comparison of S. cerevisiae 4484-24D with X2180-1A and the Heterozygous Diploid—**The mannan to phosphate ratios in strain X2180-1A and the diploid cross were higher than in 4484-24D, a result consistent with the presence of the mannosylphosphate side chains in the latter. While X2180-1A did have some phosphodiester groups in its mannan, the amount was much lower than that in the 4484-24D strain. Agglutination reactions with specific antisera supported the conclusion that the surface antigens of the diploid cross were very similar to those of the X2180-1A strain, and unlike those of strain 4484-24D (Table I). This conclusion was confirmed by the agglutination patterns (Fig. 2) which show that the diploid cross gave the

**Table I**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Agglutination of whole cells by</th>
<th>Mannose to phosphate ratio</th>
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<tr>
<td></td>
<td>Anti-X2180</td>
<td>Anti-4484-24D</td>
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<tr>
<td>X2180-1A</td>
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<td>+</td>
</tr>
<tr>
<td>4484-24D</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>X2180-1A × 4484-24D diploid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GHR10a</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>X464-20C</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>A364-4A*</td>
<td>-</td>
<td>-</td>
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</table>

*Strains GHR10 and X464-20C were used by Spencer et al. (18), while A364A was obtained from Dr. L. Hartwell and is a distant parent of 44%24D.*
nonparental ditype to tetratype for these well known genetic markers, relative to \( mnnl \), were 20:27:8 and 23:34:1. In both instances, the very low tetratype frequencies, significantly less than 67%, were diagnostic of close linkage between the \( mnnl \) gene and the centromere of some other chromosome. Therefore, additional hybrids were constructed and analyzed with a view toward assigning the marker to a map position within a specific linkage group. Noteworthy in this analysis were the tetrad ratios involving markers \( ura^3 \), \( thr^7 \), \( his^1 \), and \( arg^6 \), all assigned by earlier mapping studies to linkage group V. For the \( ura^3 \) marker which is closely linked to centromere V, we observed a tetrad ratio of 78:0:10, a distribution indicative of close gene-gene linkage. Linkage is evident from a PD:NPD:T ratio of 19:0:4 was obtained. The absence of NPD tetrads and the low tetratype frequency are findings consistent with our previous conclusions. Thus, the linkage detection and the estimates pertaining to the intensity of linkage are, as expected, independent of whether the markers exist in a coupling (cis) or repulsion (trans) phase. Since we failed to detect tetrads exhibiting an unequivocal second division segregation pattern for \( mnnl \), we cannot exclude the possibility that \( ura^3 \) and \( mnnl \) are located on opposite sides of the same centromere.

**DISCUSSION**

Chemical and immunochemical studies have demonstrated that the polysaccharide components of yeast cell wall mannans have species-specific structures, and several well characterized chemotypes have been described (11). In the present study, we attempted to define the genetic difference between two \( S. cerevisiae \) strains (Fig. 1), one characterized by the presence of a tetrasaccharide side chain (strain X2180-1A), and the other by possession of a mannosylphosphoryltri saccharide side chain (strain 4484-24D). Since each of these structures is the immunodominant group in the respective intact mannan (2, 6), we were able to use specific antisera for their detection on the cell surface. Moreover, we obtained hybrids between these two strains and studied the segregation of an allele pair that specified the mannan structure.

Several important observations were made. In the diploid hybrid of strains X2180-1A and 4484-24D, the cells displayed on their surfaces only the X2180 mannan determinant, and the isolated mannan had the properties of the X2180 strain rather than that expected of a mixture of the parental mannans. Thus, there is a simple relationship in the expression of the mannotetraose versus the mannosylphosphate determinants, with the former appearing to be dominant.

Following sporulation of the diploid hybrid and analysis of the resulting tetrads, it was found that the two mannan chemotypes segregated 2:2 in a precisely reciprocal pattern, which suggested that the structural difference was under the control of a single gene. The gene was mapped at 5.5 centimorgans proximal to \( ura^3 \) on chromosome V, tightly linked to the respective centromere. This is the first mannan gene to be placed on the yeast genetic map, and we have designated it \( mnnl \). From a consideration of the differences in mannan structure between strains X2180-1A and 4484-24D, we conclude that it is the structural gene for an enzyme involved in the addition of the \( \alpha-1 \rightarrow 3 \) linked terminal manno unit for synthesis of the mannotetraose side chain of the mannan, i.e., an \( \alpha-1 \rightarrow 3 \)-mannosyltransferase.

These results suggest that a single gene controls two phenotypic differences among the manietic segregants from the hybrid between X2180-1A and 4484-24D. Thus, a high concentration of the mannosylphosphoryltri saccharide side chain was not observed in the presence of the tetrasaccharide unit but was found when the tetrasaccharide was absent, in a homozygous diploid derived from 4484-24D and in the corresponding \( mnnl \) haploid spore clones. A simple explanation could be that, in the process of mannan biosynthesis, the \( \alpha-1 \rightarrow 3 \)-mannosyltransferase and a presumed mannosylphosphate transferase utilize the same trisaccharide acceptor, and that the former enzyme is able to compete more effectively for this substrate. Some support for this mechanism comes from a study of the properties of a different

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**Table II**

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Genetic marker</th>
<th>Tetrads scored</th>
<th>PD</th>
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<tr>
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<td>42</td>
<td>7</td>
<td>6</td>
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</table>

**Notes**

- PD = Present-Destitute
- NPD = Not Present-Destitute
- T = Total

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between \( mnnl \) and \( ura^3 \). In the original hybrids, these markers existed in a trans relation to each other, while in the recombinant, they displayed a cis configuration. Among 23 tetrads analyzed from such hybrids, a PD:NPD:T ratio of 19:0:4 was obtained.
X2180 mutant that we have obtained (15). It lacks the tetra-
saccharide side chain and presumably has a defective α-1→3-
mannosyltransferase. This mutant has a phenotype very simi-
lar to that of strain 4484-24D.

While this study reports the first precise mapping of a yeast
mannan gene, other related studies have been published. One
concerns the binding of alcian blue dye by yeast cells, which
was shown by Friis and Ottolenghi (17) to be under the control
of a single gene linked to ura1. The binding property
was located in the total mannan fraction and it appeared to be
correlated with the phosphate content of the mannan. In a
second study, Spencer et al. (18) used nuclear magnetic resonance
to follow the segregation of a terminal α-1→3-linked mannosyl
unit of the mannan in a cross between two S. cerevisiae strains,
one of which had this unit and the other which lacked it. They
found that this characteristic also was under the control of a
single gene. We suspect that this is the same gene we have
mapped, and that the two strains Spencer et al. (18) investigated
were analogous to those we have used. One of their strains
(X464-20C) was derived from S. cerevisiae S288C, the parent of
X2180; the other (GHR10), which lacks the terminal α-1→3-
mannosyl unit, has subsequently been found by us to possess
the serological properties of our strain 4484-24D (Table I). In
agreement with our results, these workers found that the gene
controlling this structural feature was not linked to ade1, his2,
his7, or ura1, and none of these is located on chromosome V.

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for assistance in ascus dissection and Dr. William C. Raschke
for help in obtaining the precipitin curves shown in Fig. 3. Mr.
Robert Becker performed some initial studies on the structure
of the mannan from S. cerevisiae 4484-24D in a laboratory class
in the Biochemistry Department. Yeast strain GHR10 was
supplied by Dr. J. F. T. Spencer, strain X464-20C by Dr. R. K.
Mortimer, and strain A364A by Dr. L. Hartwell.

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