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Genetic analysis of mutant strains of *Saccharomyces cerevisiae* with defects in mannoprotein synthesis †

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Abstract: The *mnn* mutants have constituted a fundamental tool in the study of the structure and biosynthesis of mannoproteins in Saccharomyces cerevisiae. They were isolated by the group of Dr. C.E. Ballou by random mutagenesis and a selection method using specific antibodies obtained against the wild-type strain. Initially, the mutants were characterized biochemically and in subsequent years the genes in which they were mutated were identified. All of them encode membrane proteins that catalyze the transfer of mannoses to N-oligosaccharides, sometimes isolated or as part of complexes made up of several proteins. However, the specific mutation of each of these mutants has only been identified in the case of *mnn3*. In this work we have completed the characterization of the mutants by sequencing the mutated genes in each of them. As expected, they are point mutations that involve the change of one amino acid for another in the mutated protein, or for a stop signal, resulting in a truncates protein.

Keywords: mannoprotein, *Saccharomyces cerevisiae*, *mnn* mutants, sequence.

1. Introduction

The glycosylation process in eukaryotic cells has been extensively studied during the last decades and most steps have been almost completely deciphered. In these studies, the yeast *Saccharomyces cerevisiae*, a reference model organism in many laboratories, has played a central role. The process of glycosylation of N-oligosaccharides in *S. cerevisiae* is the same as that of higher eukaryotes in the early stages, which take place in the endoplasmic reticulum (ER) which lead to the synthesis of the so-called “inner core” [1]. However, in the stages that take place in the Golgi apparatus, the so-called "outer chain" is added. These stages are different from higher eukaryotes since the outer chain contains exclusively mannose and lacks other monosaccharides. All N-linked oligosaccharides in *S. cerevisiae* are "high mannose” type. In the study of the glycosylation process in *S. cerevisiae*, the isolation and characterization of defective mutants in the process has been essential. Among them, two groups are worth highlighting: the *alg* mutants (Asparragine-linked glycosilation defective) affected in the stages of the ER [1], and *mnn* (mannan defective) affected in the stages that occur in the Golgi apparatus [2]. Figure 1 shows the defects of mnn mutants in N-oligosacharides structure. Among the latter, *mnn9* presents the most drastic defect since it blocks the addition of the whole outer chain in Golgi [2, 3]. The rest of *mnn* mutants have also been identified by different research groups working on glycosylation studies [2, 4-9]. However, the genetic defect of *mnn* mutants has only been identified in the case of the *mnn3* mutant [10].

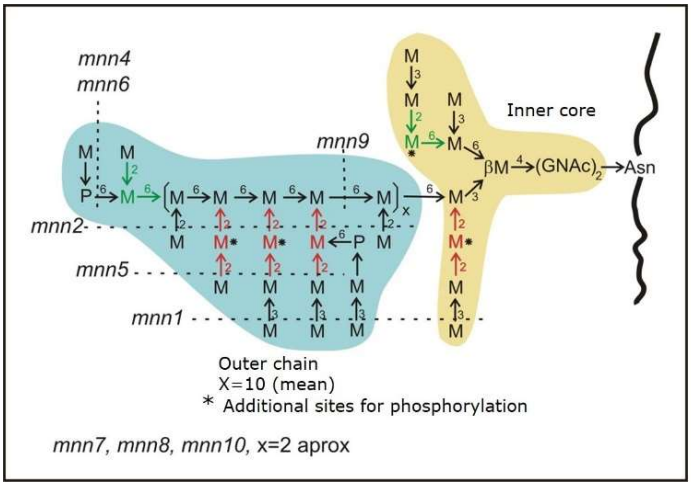

Figure 1. Chemotypes of mannoprotein mutants. Adapted from [2].

The objective of this work is to complete the genetic characterization of the *mnn* mutants, identifying the mutations in each of them, by sequencing the mutated genes in the original strains and comparing the sequences with those of the parental strain.

2. Materials and Methods

2.1. Strains

The mutants *mnn1*, *mnn2*, *mnn5*, *mnn6*, *mnn9*, *mnn10* used in this study are from the laboratory collection and were kindly supplied by Dr. C.E. Ballou some years ago. They were grown in liquid or solid YEPD medium containing 1% yeast extract, 2% peptone, 2% glucose. For solid medium, 2% agar was added.

2.2. Extraction of nucleic acids

Yeast DNA minipreps were prepared as in [11]. Yeasts cells were resuspended in 1 ml of solution I (EDTA 50mM pH 7) centrifuged and resuspended again in 1 ml of solution II (Tris-SO_4 50mM pH 9,3+1% mercaptoethanol). After 15 minutes at room temperature, they were centrifuged again and resuspended in 0.6 ml of solution III (sodium acetate 3 M). Then, 0.5 ml of equilibrate phenol is added and the mixture was incubated at room temperature for 30 min, with shaking. After centrifugation, the nucleic acids, recovered in the aqueous phase, were precipitated with 2 volumes of previously chilled isopropanol, washed with 70% ethanol, dried, and dissolved in Tris-EDTA buffer, pH 8.0.

3. Results and Discussion

Identification of mutations in MNN genes responsible for synthesis of outer chain.

Figure 2 shows the N-oligosaccharide biosynthetic pathway in the Gogi apparatus of *S. cerevisiae*. Mnn9p is part of the Mannan polymerases I and II responsible for the elongation of the backbone of mannoses linked in alpha 1-6 of the outer chain. Mnn10p is part of Mannan polymerase II, while Mnn1p, Mnn2p and Mnn5p participate in the synthesis of the side chains or branches of the main chain. Finally, Mnn6p is the transferase that catalyzes the transfer of mannose-P groups.

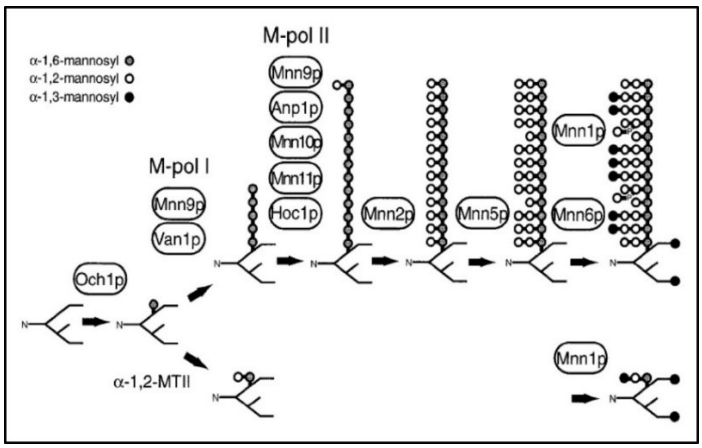

Figure 2. N-glycosylation pathway of mannoproteins in yeast Golgi [7]

GENE	Systematic name	Location in chromosome	Mutation in DNA	Effect in protein	Position in protein
MNN1	YER001W	153520..155808	G>A	C>Y	697
MNN2	YBR015C	267710..269503	G>A	G>D	359
MNN5	YJL186W	80155..81915	G>A	W>stop signal	561
MNN6	YPL053C	457118..458458	Insertion AA	Frameshift	149
MNN9	YPL050C	460779..461966	G>A	W>stop signal	16
MNN10	YDR245W	952800..953981	G>A	W>stop signal	279

Table 1. Changes in DNA sequence and effect on encoded protein, in *mnn* mutants as compared to wild type.

MNN1: located at chromosome V (YER001W), 2289 bp. It encodes an integral membrane protein with alpha-1,3-mannosyltransferase activity. The mutation in *mnn1* is a missense point mutation that changes G by A in DNA. It results in a change of cysteine by tyrosine in the protein.

MNN2: located ay chromosome II (YBR015C), 1794 bp. It encodes an integral membrane protein with alpha-1,2-mannosyltransferase activity over mannoses linked by alpha-1,6. The mutation in *mnn2* is a missense point mutation that changes G by A in DNA. It results in a change of glycine by aspartic acid in the protein.

MNN5: located at chromosome V(YJL186W), 1761 bp. It encodes an integral membrane protein with alpha-1,2-mannosyltransferase activity over some mannoses linked by alpha-1,2. The mutation in *mnn5* is a nonsense point mutation that changes G by A in DNA. It results in a change of tryptophan by a stop signal the protein, thus resulting in a truncated protein.

MNN6: located at chromosome XVI (YPL053C), 1341 bp. It encodes an integral membrane protein with mannosylphosphate transferase activity. The mutation in mnn6 is a frameshift mutation due to insertion of AA. The change in the reading frame most probably results in a nonfunctional protein.

MNN9: located at chromosome XVI (YPL050C), 1188 bp. It encodes an integral membrane protein which is part of a complex involved in outer chain elongation. The mutation in *mnn9* is a nonsense point mutation that changes (G by A) in DNA. It results in a change of tryptophan by a stop signal the protein, thus resulting in a truncated protein.

MNN10: located at chromosome IV (YDR245W), 1182 bp. It encodes an integral membrane protein which is part of a complex involved in outer chain elongation. The mutation in *mnn10* is a nonsense point mutation that changes G by A in DNA. It results in a change of tryptophan by a stop signal the protein, thus resulting in a truncated protein.

As expected, in most cases are point mutations with different effects: missense mutations, nonsense mutations or frameshift mutations.

In the last few years, the mnn mutants have been widely characterized phenotypically. With this study, the genotypic characterization is completed.

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Conflicts of Interest: "The authors declare no conflict of interest."

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