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An *MFα1–SUC2* (α-factor–invertase) gene fusion for study of protein localization and gene expression in yeast

(recombinant DNA/transcriptional regulation/secretion/proteolytic processing/protein glycosylation)

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ABSTRACT **The peptide mating pheromone α-factor and the hydrolytic enzyme invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) are processed from larger precursor proteins during their secretion from yeast cells (*Saccharomyces cerevisiae*). An in-frame fusion of the structural genes for these two proteins was constructed by connecting the 5′-flanking region and prepro-leader portion of the coding sequence of the α-factor gene (*MFα1*) to a large fragment of the invertase gene (*SUC2*) lacking its 5′-flanking region and the coding information for the first four amino acids of its signal sequence. Sites that have been implicated in normal proteolytic processing of the α-factor precursor have been retained in this construction. The chimeric gene directs synthesis of a high level of active invertase that is secreted efficiently into the periplasmic space, permitting cell growth on sucrose-containing media. This extracellular invertase appears to contain no prepro-α-factor sequences. The initial intracellular product is, however, a hybrid protein that can be detected either by treatment of the cells with the drug tunicamycin or by blockage of secretion in a temperature-conditional secretion-defective mutant (*sec18*). Therefore, prior to its efficient proteolytic removal, the α-factor portion of the hybrid protein apparently provides the necessary information for efficient export of the substantially larger protein invertase. Similar to *MFα1*, the *MFα1–SUC2* fusion is expressed in α haploids at levels 65–75 times higher than in a haploids or in a/α diploids; also, high-level expression is eliminated in *matα1* mutants but not in *matα2* mutants. Unlike expression of *SUC2*, expression of the fusion is not affected by glucose concentration. Hence, the 5′-flanking region present in the fusion (about 950 base pairs) is sufficient to confer α cell-specific expression to the hybrid gene.**

Fusions of bacterial genes to the *lacZ* gene of *Escherichia coli* have been extremely useful for determining the level of expression of gene products that otherwise would be difficult to detect because there are convenient methods for monitoring the activity of β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) (1, 2). This approach has been extended to analyze the regulation of expression of several yeast genes (3–7). Studies in *E. coli* (8, 9) and more recently in yeast (unpublished data) demonstrate that β-galactosidase is unable to be translocated through a membrane, even when coupled to secretory proteins. In *E. coli*, this property results in certain characteristic phenotypes that have been used to select mutations that have permitted the genetic dissection of the process of prokaryotic protein export (10, 11). In yeast, however, this feature of β-galactosidase might limit its utility in the analysis of eukaryotic secretory transport (which includes protein translocation into the endoplasmic reticulum, delivery to the Golgi complex, protein sorting, packaging into vesicles, and final targeting to unique cellular destinations). To overcome this limitation, but to take advantage nonetheless of the utility of gene fusions for studying protein secretion and gene regulation in yeast cells, we sought a different and more appropriate indicator enzyme for use in constructing gene fusions.

The *SUC2* gene of yeast codes for the enzyme invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) (12), which is required for growth on sucrose as sole carbon source. This gene and its product have several biochemical and genetic advantages that should permit development of a generally applicable gene fusion system for yeast.

The *SUC2* gene has been cloned (13) and its entire nucleotide sequence has been determined (14). Invertase itself is a very stable dimeric protein that is secreted via the yeast secretory pathway (15, 16). The secreted molecule bears long, N-linked, mannose-rich oligosaccharide chains (17) on at least 9 of its 13 potential glycosylation sites (14, 18, 19). The enzyme is too large to pass through the yeast cell wall and remains trapped in the so-called periplasmic space. Extracellular invertase is produced from a transcript that codes for a precursor containing a 19-residue hydrophobic signal sequence (20, 21). Synthesis of the external form is repressed by the presence of glucose because transcription of the gene is reduced and only a short transcript, which does not encode the signal sequence, is made (13). Thus, a low level of an internal form is made. Invertase activity present in whole cells, either intact or made permeable by various methods, can be determined through simple and sensitive colorimetric assays that measure either the glucose or the fructose released upon sucrose hydrolysis (22). Furthermore, these assays can be adapted to be performed directly on colonies on a solid medium or on filter paper replicas of such clones (ref. 23; unpublished results). Finally, growth on sucrose provides a direct genetic selection for functional invertase.

We chose the structural gene *MFα1*, which encodes the 165-amino acid precursor of the yeast mating pheromone, α-factor (αF), to determine how successful gene fusions to *SUC2* might be. αF is a peptide hormone-like effector that is secreted only by α haploids and is required (24) to prime haploid yeast cells of the opposite mating type, a cells, for eventual conjugation with α cell partners (for review, see ref. 25). The *MFα1* gene has been cloned and its entire nucleotide sequence has been determined (26, 27). Because only *MATα* cells contain a mRNA complementary to *MFα1* (27), expression of the gene is apparently under the direct transcriptional control of the mating type locus (28). αF is excised from its larger precursor, prepro-αF (27, 29), which contains four tandem copies of the pheromone (26, 27). The precursor enters the secretory system, is glycosylated with short core oligosaccharides at three sites (un-

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Abbreviations: αF, yeast α-factor mating pheromone; bp, base pair(s).

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published data), and is processed at a late stage in the secretory pathway by both endoproteolytic and exoproteolytic cleavages (29) to yield the mature pheromone. Unlike processing of the secreted form of invertase, the amino-terminal hydrophobic leader of 20 or so residues is apparently not removed during translocation of prepro-αF into the lumen of the endoplasmic reticulum (unpublished data).

In this report, we describe the construction of a *MFα1–SUC2* gene fusion, demonstrate the cell type-specific regulation of its expression, and characterize the processing and secretion of its hybrid protein product.

MATERIALS AND METHODS

Yeast and Bacterial Strains. Genetic crosses (30) were used to construct the following *Saccharomyces cerevisiae* strains, each of which contains a complete deletion (*suc2-Δ9*) of the chromosomal copy of *SUC2* (C. Falco and M. Carlson, personal communication) and contains no other unlinked invertase structural gene (*SUC1*, *SUC3-SUC7*) (13): SEY2101, *MATa ura3-52 leu2-3,-112 ade2-1*; SEY2102, *MATα ura3-52 leu2-3,-112 his4-519*; SEYD2112, *MATa/MATα ura3-52/ura3-52 leu2-3,-112/leu2-3,-112 +/ade2-1 +/his4-519*; SEY5188, *MATα sec18-1 ura3-52 leu2-3,-112*; and XMF40-3a, *matα1*-ochre *ura3-52 leu2 trp1-289 lys1-1*. Strain XBH15a-34c, *matα2 SUC2 ura3-52 leu2-3,-112 his4-580 trp1-289* (from L. C. Blair), was used in certain experiments. Bacterial transformations were performed with the following *E. coli* K-12 strains: JM83, F⁻ *ara Δ(lac–pro) rpsL thi* [*φ80dlacZΔM15*] (from J. Messing); MC1061, F⁻ *hsdR⁻ hsdM⁺ araD139 Δ(araABOIC–leu)7679 Δ(lac)X74 galU galK rpsL*; and MC1066, F⁻ *hsdR⁻ hsdM⁺ pyr*F::Tn5 *Δ(lac)X74 galU galK rpsL trpC9830 leuB600* (from M. Casadaban).

Plasmid Vectors and Recombinant DNA Methodology. pBR322 (31), pUC8 (32), YEp24 (33), pAB101 (27), and pRB58 (13) have been described previously. pCGS139 was the gift of G. Vovis. Restriction endonuclease digestions and ligations with phage T4 DNA ligase were conducted as recommended by the suppliers. Plasmid purifications, agarose gel electrophoresis, DNA-mediated transformations of yeast and bacteria, and other manipulations of nucleic acids were performed by standard methods (30, 34, 35).

Enzyme Assay. Invertase activity was measured as the glucose generated from sucrose hydrolysis by minor modifications (36) of the colorimetric procedure of Goldstein and Lampen (22). All values given represent the average of duplicate determinations and were the results of measurements performed under conditions in which the amount of glucose generated was linear with respect to both time and the number of cells added. *In situ* staining for invertase activity in nondenaturing polyacrylamide gels has been described (15, 37).

Radiolabeling and Immunoprecipitation. Labeling of yeast cells with $[^{35}S]SO_4^{2-}$ in low-sulfate medium in the presence and absence of tunicamycin (Sigma), subcellular fractionation of the radioactive cells and removal of the cell wall by lytic enzyme (38) digestion, immunoprecipitations with anti-invertase antibody (gift of I. Schauer), and electrophoresis of proteins in slabs of polyacrylamide gel in the presence of $NaDodSO_4$ have all been described (15, 39). To remove N-linked glycosyl chains, protein samples were resuspended in a final volume of 50 μl of 50 mM 2-mercaptoethanol/0.5% $NaDodSO_4$/5 mM NaN_3/100 μg of bovine serum albumin per ml/0.27 M sodium citrate, pH 5.5, boiled for 3 min, cooled to ambient temperature, incubated with 12.5 ng of endoglycosidase H (40) (gift of P. Robbins) for 10–12 hr at 37°C, subjected to a second boiling, and redigested with 6 ng of endoglycosidase H for 10–12 hr at 37°C. After this treatment, the reaction mixtures were dialyzed exhaustively against water, lyophilized, and resuspended in electrophoresis buffer (15, 39).

RESULTS

Construction of the *MFα1–SUC2* Gene Fusion. The coding sequence for the amino-terminal prepro-leader portion (89 amino acids) of the αF precursor (165 amino acids) was fused to almost the entire coding sequence (528 amino acids) of the precursor (532 amino acids) to secreted invertase (513 amino acids) (Fig. 1). The coupling results in the in-frame fusion of the two coding segments. Preserved at the fusion junction is the presumed site (-Lys-Arg-) for endoproteolytic processing that precedes each copy of pheromone sequence in the αF precursor (Fig. 2). Pairs of basic residues have been implicated as processing sites for the excision of many different peptide hormones from their precursors in other eukaryotic systems (41). In addition, the repeating -Glu-Ala- sequence known to be required for exoproteolytic processing of pro-αF by dipeptidyl aminopeptidase A (29) also is retained in the fusion. A DNA fragment containing the entire fusion was excised with *Eco*RI and *Pvu* II and inserted into an appropriate shuttle plasmid (Fig. 1).

Expression of Invertase Activity from the *MFα1–SUC2* Gene Fusion. No invertase activity was detectable in *E. coli* cells harboring either pSE210 or pSEY210. In marked contrast, when yeast *MATα* cells deleted for their chromosomal copy of *SUC2* were transformed with pSEY210, a high level of invertase activity was found (Table 1).

If yeast cells carrying a plasmid with a 2-μm circle *ori* are propagated nonselectively, segregational loss of the plasmid occurs quite frequently (34). When the transformants were cured of pSEY210 in this way, every uracil-requiring segregant tested also had lost detectable invertase activity. Release of cell wall material from α cells transformed with pSEY210 by lytic enzyme digestion in osmotically stabilizing medium demonstrated that over 90% of the invertase activity was present in the extracellular periplasmic fraction, indicating that invertase had been efficiently secreted in these cells. This will be documented further in the next section.

Normal invertase expression from the *SUC2* gene is subject to glucose repression (Table 1). In the case of cells transformed with pSEY210, however, the level of invertase expression was completely unaffected by the concentration of glucose in the medium. Invertase expression from pSEY210 appears to mimic the pattern of regulation expected for the normal *MFα1* gene. The fusion gene is expressed in α cells at levels 65–75 times higher than in a cells or in a/α diploids (Table 1). This same pattern was seen upon transformation of several independently derived sets of a, α, and a/α diploid strains.

Both genetic (28) and biochemical (42) evidence supports the view that expression of α-specific functions requires the product of the *MATα1* gene, but not the product of the other gene coded for at *MATα*, *MATα2*. Hence, as another test that regulation of expression of the fusion was behaving similarly to that of the normal *MFα1* gene, yeast strains bearing either a *matα1* mutation or a *matα2* mutation were transformed with pSEY210. As anticipated, high-level expression of invertase activity was eliminated in the *matα1* background but not in the *matα2* background (Table 1).

The *MFα1-SUC2* Gene Fusion Directs the Synthesis of a Hybrid Protein. The fusion gene carried by pSEY210 should direct the synthesis of a hybrid protein that contains prepro-αF sequences at its amino terminus and invertase sequences at its carboxyl terminus. We labeled cells with $[^{35}S]SO_4$, lysed them, immunoprecipitated invertase-related proteins from various subcellular fractions by using anti-invertase antibodies, and

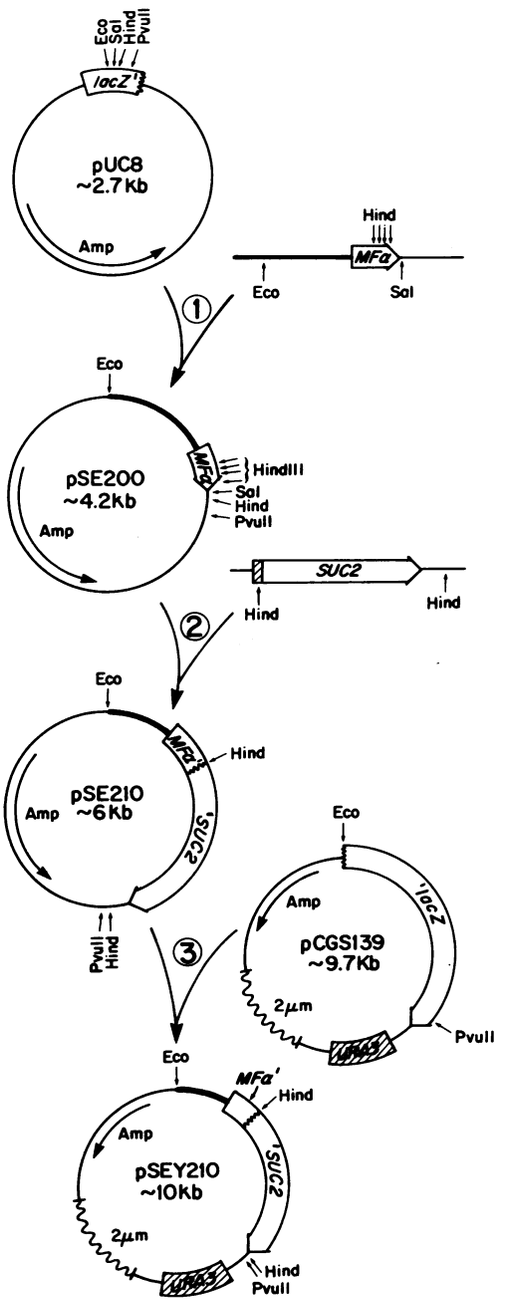

FIG. 1. Construction of the *MFα1–SUC2* gene fusion. Only those restriction endonuclease cleavage sites that are pertinent to the construction scheme are indicated. (*1*) A fragment of DNA derived from pAB101 (27) extending from an *Eco*RI site 950 base pairs (bp) upstream from the initiation codon of the *MFα1* coding region to a *Sal* I site 37 bp downstream from the termination codon of the coding region was ligated into the *E. coli* cloning vehicle pUC8 that had been digested with both *Eco*RI and *Sal* I, yielding pSE200. Insertion into this site places short fragments of *lacZ* DNA both upstream and downstream from the *MFα1* segment. Amp, ampicillin; kb, kilobase pairs. (*2*) pSE200 was digested with *Hin*dIII, treated with bacterial alkaline phosphatase, and then ligated to a DNA fragment, derived from pRB58 (13) by *Hin*dIII cleavage, that contains coding information for 15 of the 19 amino acids of the signal sequence and for the rest of the 513 amino acids of the mature invertase polypeptide, as well as about 500 bp of DNA downstream from the termination codon of the *SUC2* gene. A derivative that contained the *SUC2* sequence in the same orientation as the *MFα1* sequence was identified by restriction endonuclease mapping and designated pSE210. Short stretches of *lacZ* DNA also flank the fusion in this plasmid. (*3*) The *Eco*RI/*Pvu* II DNA fragment within pSE210 that contains the *MFα1–SUC2* chimeric gene was then ligated into an appropriate yeast episomal shuttle vector, pCGS139, that had been digested with both *Eco*RI and *Pvu* II, yielding pSEY210. Transformants harboring pSEY210 displayed only a low level of ampicillin resistance for unknown reasons. Only a short segment of *lacZ* DNA at the 3′ end of the fusion is retained in pSEY210. 2 μm, a yeast plasmid.

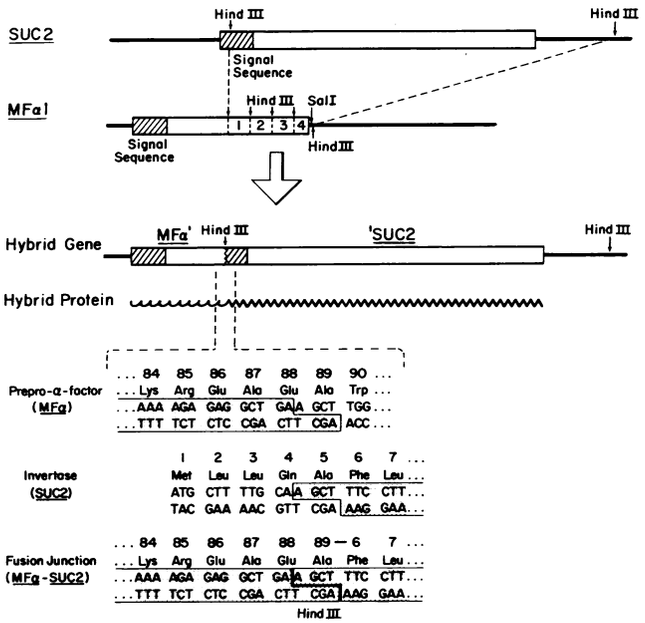

FIG. 2. Basic features of the *MFα1–SUC2* gene fusion. A cleavage site for *Hin*dIII restriction endonuclease within the signal sequence of the precursor for secreted invertase is in the same reading frame as the *Hin*dIII site that just precedes each of the four tandem repeats of αF coding information within the *MFα1* gene. The predicted DNA sequence and corresponding amino acid sequence of the junction region are shown in detail. Each rectangular box designates a protein coding region and the hatched area represents residues corresponding to its hydrophobic signal sequence.

analyzed the immunoprecipitates by electrophoresis in slabs of polyacrylamide gel containing $NaDodSO_4$. Two types of strains transformed with pSEY210 were examined: a normal *MATα* strain (SEY2102) containing the *suc2-Δ9* deletion and a derivative (SEY5188) that also carried a temperature-sensitive mutation (*sec*18-1) that causes the accumulation of core-glycosylated secretory proteins within the lumen of the endoplasmic reticulum when the cells are shifted to the restrictive temperature (15). As controls, both of these yeast strains were also transformed with pRB58, which carries the normal *SUC2* gene.

In normal cells transformed with either *SUC2* or the fusion gene, a very heterodisperse mixture of highly glycosylated forms that are characteristic of secreted invertase were found in the periplasmic fraction (Fig. 3). The majority of the species produced by cells carrying the *SUC2* plasmid had molecular weights in the range 100,000–125,000, whereas the bulk of those produced by cells carrying the chimeric gene were in the range 75,000–91,000. The glycosyl chains were removed from this extracellular material by exhaustive treatment with endoglycosidase H (40). The protein portion of the molecules produced by either the *SUC2*- or the fusion-containing cells had the same apparent molecular weight, about 57,000–58,000. This value agrees well with the size expected for a single chain of mature invertase (58,567) predicted from the DNA sequence of the gene (14).

Tunicamycin is a drug known to block the synthesis of N-linked oligosaccharide chains (43) and to cause, in certain cases, the accumulation of unglycosylated secretory proteins within the lumen of the endoplasmic reticulum. When normal cells carrying the two different plasmids were labeled in the presence

Table 1. Expression of the *MFα1–SUC2* gene fusion is under *MATα* control

Strain*	Plasmid†	% glucose in medium‡	Enzyme activity§
MATα suc2-Δ9	YEp24	2	<0.1
MATα suc2-Δ9	p[*MFα1–SUC2*]	2	810
MATa suc2-Δ9	p[*MFα1–SUC2*]	2	12
MATa/MATα suc2-Δ9/suc2-Δ9	p[*MFα1–SUC2*]	2	10
MATα suc2-Δ9	p[*MFα1–SUC2*]	0.1¶	775
MATα suc2-Δ9	p[*MFα1–SUC2*]	6¶	765
MATα suc2-Δ9	p[*SUC2*]	0.1¶	1,020
MATα suc2-Δ9	p[*SUC2*]	6¶	60
matα2 SUC2	p[*MFα1–SUC2*]	6	930
matα2 SUC2	YEp24	6	5
matα1 suc2-Δ9	p[*MFα1–SUC2*]	2	15

* Only the relevant genetic markers of the strains listed in *Materials and Methods* are given.

† p[*MFα1–SUC2*] = pSEY210, p[*SUC2*] = pRB58, and YEp24 is a control vector that contains no *SUC2* sequences.

‡ All cultures were grown at 24°C to an optical density of 0.8–1.0 at 600 nm in minimal medium (15) with required supplements, but lacking uracil to maintain selection for the plasmids, and were washed thoroughly in two changes of 4 ml each of 10 mM NaN_3 prior to invertase assay.

§ External invertase activity was measured with whole cells and is expressed as nmol of sucrose hydrolyzed per min per OD_{600} of cells. As expected from the previous work of others (12, 13), the level of invertase activity produced by *SUC2* strains, or by *suc2* strains carrying the *SUC2* gene on a plasmid, was the same regardless of the mating type of the cells (results not shown).

¶ In this experiment, cells were pregrown in 6% glucose and then shifted to medium containing either 0.1% or 6% glucose for 3 hr prior to assay.

of tunicamycin, the fusion gene produced a protein of higher apparent molecular weight (about 66,000) than that produced by *SUC2* itself (about 56,000) (Fig. 3). Indeed, the expected increase in molecular weight due to the additional presence on invertase of the prepro-leader portion of the αF precursor can be predicted from the amino acid sequence of the *MFα1* gene (26, 27) and is about 10,000.

To confirm that the hybrid protein is present intracellularly and that its transport follows the secretory pathway traversed by invertase itself, *sec18* mutant cells carrying both plasmids were labeled at the nonpermissive temperature. Under these conditions, no labeled invertase could be detected outside the cells (results not shown). As anticipated, multiple discrete species of higher molecular weight than those accumulated in the presence of tunicamycin were observed inside the cells (Fig. 3). These bands presumably represent molecules to which different numbers of core oligosaccharides have been added because removal of the glycosyl chains by endoglycosidase H treatment produced single species very similar in molecular weight to those observed upon labeling of normal cells in the presence of tunicamycin.

To determine if the hybrid protein itself possessed functional invertase activity, it was accumulated intracellularly by shifting *sec18* mutant cells carrying the fusion plasmid to the restrictive temperature. The cells were converted to spheroplasts by treatment with cell wall lytic enzyme in an osmotically stabilizing medium. The spheroplasts were collected, washed thoroughly, and lysed by treatment with the nonionic detergent Triton X-100. The lysate was subjected to electrophoresis in a polyacrylamide gel under nondenaturing conditions. Staining for invertase activity *in situ* revealed that the hybrid protein has activity and runs with an altered mobility in comparison to the

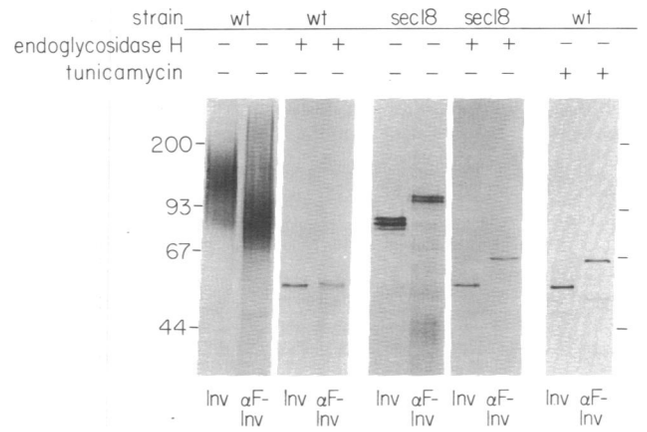

FIG. 3. The *MFα1–SUC2* fusion directs the synthesis of a hybrid protein. Cultures (4 ml) of wild-type (wt) cells (SEY2102) transformed with either pRB58 (lanes designated Inv, for invertase) or with pSEY210 (lanes designated αF-Inv) were grown at 24°C in low-sulfate medium (100 μM) lacking uracil to an OD_{600} of 0.8–1 and then pulse-labeled in minimal medium (2 ml) with a final concentration of $[^{35}S]SO_4$ of 800 μCi/ml and 10 μM total sulfate for 30 min. The cells were harvested by centrifugation, washed once in an equal volume of 10 mM NaN_3, and lysed by either of two techniques, depending on the experiment and the particular subcellular fraction to be examined. In some cases, cells were broken by vigorous Vortex mixing with glass beads. In the analysis shown here, cells were first converted to spheroplasts and then separated from the cell wall and periplasmic fraction by brief centrifugation. The spheroplasts were lysed by boiling in 1% $NaDodSO_4$ (25 μl). Samples of both the lysate and the cell wall/periplasmic fraction were then diluted with 2% Triton X-100 in phosphate-buffered saline solution (800 μl) and subjected to immunoprecipitation (15, 39). In some experiments, cells were treated with tunicamycin at a final concentration of 12 μg/ml for 15 min prior to labeling. Samples of the immunoprecipitated material were subjected to electrophoresis in slabs of 8% polyacrylamide gel containing $NaDodSO_4$, either before or after treatment with endoglycosidase H (40). Similar experiments were performed with cells (SEY5188) bearing the temperature-sensitive *sec18* mutation (*sec18*) that carried either pRB58 (Inv) or pSEY210 (αF-Inv); however, pulse-labeling was initiated after the cultures had been shifted to the nonpermissive temperature (37°C) for 10 min. See text for further explanation. Numbers are molecular weight × 10^{-3} of marker proteins.

control invertase produced by *SUC2* plasmid-containing cells treated in the same manner (results not shown).

DISCUSSION

An objective of this work was to determine if the *SUC2* gene, which codes for a well-characterized, readily detectable, and efficiently secreted yeast enzyme, would provide a useful indicator for constructing gene fusions to study both the localization and the regulation of expression of other proteins in yeast. We constructed successfully a functional fusion of *SUC2* to the structural gene for the αF precursor, *MFα1*. The simplest interpretation of our results is to assume that the prepro-leader of the αF precursor is itself sufficient to direct the efficient export of invertase, which is a significantly larger protein than the remainder of the pheromone precursor itself. Nonetheless, because about two-thirds of the normal invertase signal sequence remains in the fusion as the result of the manner of its construction, it is possible that the presence of these residues assists in the translocation of the hybrid protein through the membrane of the endoplasmic reticulum. This seems somewhat unlikely, however, because these hydrophobic residues are separated by a substantial distance (89 amino acids) from the amino terminus of the hybrid protein. Furthermore, it appears that the prepro-leader region of the αF precursor is competent to direct the efficient secretion of quite a number of heterol-

ogous (non-yeast) proteins that have been fused to it, including epidermal growth factor (A. Brake, personal communication), interferon (A. Singh, personal communication), and β-endorphin (G. Bitter, personal communication). It should be noted, however, that all of these molecules are normally secreted products. It is not yet known whether the leader region of prepro-αF will permit the export of any typical cytoplasmic polypeptide.

The protein portion of the invertase secreted by cells carrying the fusion gene is apparently similar in size to that of the normal enzyme. This finding suggests that the proteolytic processing system responsible for maturation of the αF precursor operates efficiently on the hybrid polypeptide. Interestingly, the extracellular invertase produced from the fusion gene is somewhat underglycosylated. Possibly, the prepro-leader region of the αF precursor, which normally receives only core oligosaccharides (unpublished data), remains as part of the hybrid after its entry into the secretory system for a sufficient period to reduce the rate or extent of polymerization of the longer mannose-rich outer chains that are added to the core oligosaccharides of invertase.

Upon initial translocation of the chimeric molecule into the lumen of the endoplasmic reticulum, it appears that signal peptidase does not cleave within the segment of the invertase signal sequence present in the hybrid because the hybrid molecule accumulates intact in *sec*18 mutants arrested at the restrictive temperature. Invertase itself has its signal sequence removed by this stage in the secretory pathway, as demonstrated by the migration of the endoglycosidase H-treated extracellular molecules with the endoglycosidase H-treated species accumulated in the endoplasmic reticulum in *sec*18 mutants (Fig. 3).

Regulation of expression of the invertase activity produced by the fusion reflects *MATα* control of the *MFα*1 gene rather than normal control of *SUC*2 itself (Table 1). The *MFα*1–*SUC*2 fusion should have considerable utility for dissecting the *MFα*1 promoter because effects on expression of deletions and other alterations generated *in vitro* can be monitored by following invertase activity. The fusion gene also should permit the development of convenient tests for identifying mutations *in vivo* that alter either expression of the gene or the proper localization and secretory transport of its product. In a similar way, fusion of *SUC*2 to the promoters or coding sequences of other genes, particularly those specifying proteins that are translocated into or through membrane-bounded cellular compartments, may prove generally useful in the study of the molecular and cellular biology of yeast cells.

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