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Synthesis of *Saccharomyces cerevisiae* invertase by *Schizosaccharomyces pombe*

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In order to gain information on the ability of the glycosylation system of *Schizosaccharomyces pombe* to process heterologous glycoproteins, the expression of *Saccharomyces cerevisiae* invertase in the former yeast was studied. *Sc. pombe* cells are able to produce enzymatically active invertase from the *S. cerevisiae SUC2* gene introduced by transformation and the enzyme is glycosylated and secreted into the cell wall. However, *Sc. pombe* transformants do not glycosylate the heterologous enzyme as their own invertase since it is not bound by the lectin from *Bandeiraea simplicifolia* seeds, which indicates the absence of terminal galactose residues. Moreover, the electrophoretic mobility of the heterologous invertase is similar to that of the large enzyme from *S. cerevisiae*, both in its native form and after being deglycosylated with Endo H. These results suggest that the polypeptide chain of *S. cerevisiae* invertase is the primary factor for the glycosylation in *Sc. pombe* cells.

Yeast; Invertase; Glycoprotein; Glycosylation; Transformation; Heterologous gene expression

1. INTRODUCTION

Although *Saccharomyces cerevisiae* has been widely used as a host for the expression of recombinant DNA molecules, this yeast has several disadvantages for the functional expression of heterologous eukaryotic genes [1–4]. One such handicap derives from the different glycosylation systems that work in *S. cerevisiae* and higher eukaryotes, which is one of the reasons for the improper processing and secretion of heterologous products by this yeast [5,6].

To evaluate the usefulness of *Schizosaccharomyces pombe* as an alternative to *S. cerevisiae* for the expression of heterologous glycoproteins, we studied the synthesis of a well-known glycoprotein enzyme from *S. cerevisiae*, invertase, in *Sc. pombe*.

S. cerevisiae cells contain two different forms of invertase: a 'large' glycosylated enzyme, which is secreted into the cell wall, and a 'small' unglycosylated form which remains in the cytosol; both forms are synthesized from different mRNAs which are transcribed from the same structural gene [7,8]. The large enzyme contains 9–10 N-linked oligosaccharides of the high-mannose type [9] and its synthesis is regulated by catabolite repression [10].

Sc. pombe invertase is also a glycoprotein associated with the cell wall which differs from the *S. cerevisiae* large enzyme in two important aspects: a much larger molecular mass and the presence of terminal residues of galactose in the sugar moiety [11]. The synthesis of *Sc. pombe* invertase is also regulated by glucose but under repression conditions the cells maintain a basal enzyme level [12].

Although it has recently been shown that the

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Abbreviations: Con A, concanavalin A; Endo H, endo-β-*N*-acetylglucosaminidase H

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glycosylation of *S. cerevisiae* invertase in mouse fibroblasts is controlled by host-dependent factors [13], in the present paper we offer results which seem to indicate that the processing of the asparagine-linked oligosaccharides of the enzyme in *Sc. pombe* is regulated by the primary structure of the polypeptide chain.

2. MATERIALS AND METHODS

2.1. *Strains, plasmids and culture conditions*

The invertase-deficient mutant of *Sc. pombe* CY-239 (*ura*4-294, *inv*3, h^-) was obtained by treatment of the wild-type haploid strain 972 h^- with ethyl-methane sulfonate followed by standard genetic techniques. Strain *ura*4-294 was also constructed by standard genetic procedures from strains 972 h^- and *ura*4-294, *ade*6-250, *arg*1-131, h^+, both obtained from Dr P. Munz (University of Bern, Switzerland).

The wild-type haploid strain of *S. cerevisiae* X2180-1A was obtained from the Yeast Genetics Stock Center (Berkeley, CA).

The yeast multicopy plasmid pRB58, a YEp24-derived 2 μ plasmid containing the *S. cerevisiae SUC*2 gene for invertase, was obtained from Dr M. Carlson and has been described elsewhere [7].

Liquid cultures were grown in YEPD medium, containing 2% yeast extract (Difco), 1% peptone (Difco) and 2% glucose, at 30°C, on a rotary shaker at 250 rpm. Transformants of *ura*4-294 and CY-239 strains with plasmid pRB58 were grown in a minimal medium, containing 0.67% yeast nitrogen base without amino acids (Difco) and 2% glucose, with appropriate nutritional supplements at 50 μg/ml. To obtain cells derepressed for invertase synthesis the glucose concentration in the media was reduced to 0.1%.

2.2. *DNA preparation and transformation procedure*

Plasmid DNA was prepared by following the procedures described by Godson and Vapnek [14] and Maniatis et al. [15].

Sc. pombe strains were transformed with plasmid DNA according to the method of Beach et al. [16], except that spheroplasts were obtained by incubation in Novozym 234 at a final concentration of 2 mg/ml.

2.3. *Preparation of cell lysates*

Cells from a 200 ml liquid culture grown to early exponential phase (about 1 A_{600} unit of cells) were harvested by low speed centrifugation and washed twice with 0.1 M acetate buffer, pH 4.0. Cell disruption was performed by mixing 1 vol. of cells with two vols of acid-washed glass beads (0.3–0.5 mm in diameter) and shaking vigorously on a Vortex-mixer for 4 min at 4°C. The cell extract was diluted with 1 ml of the former buffer and the beads and unbroken cells were removed by centrifugation at 4000 × *g* for 5 min. The supernatant was finally spun down at 12000 × *g* for 10 min to obtain the soluble fraction used for invertase assays and electrophoretic analyses.

2.4. *Invertase assay*

Invertase activity was measured at 30°C as described by Goldstein and Lampen [17], except that the pH of the reaction mixture was 4.0. One unit of invertase hydrolyzes sucrose releasing 1 μmol of glucose per min at 30°C and pH 4.0.

2.5. *Polyacrylamide gel electrophoresis*

Electrophoresis was performed in 3–10% polyacrylamide linear-gradient gel slabs according to the method of Hames [18] except that SDS was omitted. Samples were prepared by mixing 50 μl of cell extract, containing 0.1–0.5 units of invertase, and 50 μl of 0.125 M Tris-HCl buffer, pH 6.8, containing 20% Ficoll and 1 mg bromophenol blue/ml.

Invertase activity was detected 'in situ' following the method of Gabriel and Wang [19], as modified by Carlson et al. [20].

2.6. *Other methods*

Deglycosylation of invertase with Endo H was performed under the conditions described in [21].

Precipitation of invertase with lectins was carried out as previously described [11].

2.7. *Chemicals*

Reagents for gel electrophoresis were obtained from Bio-Rad. Glucose oxidase, peroxidase, *o*-dianisidine, polyethylene glycol, 2,3,5-triphenyltetrazolium chloride, Con A-Sepharose and the lectin from *Bandeiraea simplicifolia* seeds were from Sigma. Novozym 234 was purchased from Novo Industri As. (Copenhagen, Denmark) and Endo H was obtained from Miles Scientific. All other reagents were of analytical grade.

3. RESULTS

3.1. *Transformation of* Sc. pombe *with the* SUC2 *gene from* S. cerevisiae

The *Sc. pombe* strains *ura*4-294 and CY-239 were transformed with the plasmid pRB58 under the conditions described in section 2. The former strain shows a wild-type phenotype for invertase production while the latter carries the *inv*3 mutation which prevents derepression of invertase synthesis, maintaining the enzyme in the cells at basal levels (results to be described in detail elsewhere); the *ura*4-294 mutation in both strains is complemented by the *S. cerevisiae URA*3 gene [22], contained in the pRB58 plasmid along the structural gene for invertase, *SUC*2. Consequently, transformants were selected according to their Ura^+ phenotype and later by their increased levels of invertase when growing under repressing conditions (media with 3% glucose) for synthesis of the enzyme. 100–200 transformants per μg of DNA were obtained as well as a large number of abortive microcolonies indicating that the plasmid replicates deficiently in *Sc. pombe*.

As shown in table 1, the invertase activity in whole cells from the transformants was about 10–15 times higher than that observed in control

Table 1

Invertase activity in *Sc. pombe* transformants

Strain	Plasmid (pRB58)	Invertase (U/100 mg cells) in	
		Whole cells	Cell extracts
972 h⁻	–	1.0	0.95
	+	15.0	16.3
CY-239	–	1.7	2.1
	+	17.2	18.6

Experimental details are described in the text

strains without plasmid and a similar result was obtained when the activity was determined in cell extracts.

3.2. *Characterization of the heterologous invertase produced by* Sc. pombe *transformants*

The apparent size of the invertase produced by the transformants was examined by polyacrylamide gel electrophoresis under non-denaturing conditions. Fig.1 shows that the electrophoretic mobility of the heterologous invertase was similar to that of the large enzyme from *S. cerevisiae* which, together with the diffuse pattern of its band, seemed to indicate that the enzyme synthesized from the *SUC2* gene was being glycosylated in the transformants. This latter assumption was confirmed by lectin-binding experiments and treatment with Endo H.

Table 2 shows that most of the invertase produced by the transformants was bound to Con A in a similar way to the enzymes produced by the wild-type strains from *Sc. pombe* and *S. cerevisiae*. However, only the invertase produced by the wild-type strain from *Sc. pombe* was precipitated by the lectin from *Bandeiraea simplicifolia* seeds, indicating that the enzyme synthesized by the transformants was glycosylated in a different manner.

When cell extracts from the transformants were treated with Endo H a drastic change in the electrophoretic mobility of the invertase was obtained. As shown in fig.2, the deglycosylated heterologous invertase from the transformants moved into the gel to a position quite similar to that of the *S. cerevisiae* large enzyme and clearly different to that of the *Sc. pombe* 'genuine' invertase. Similar

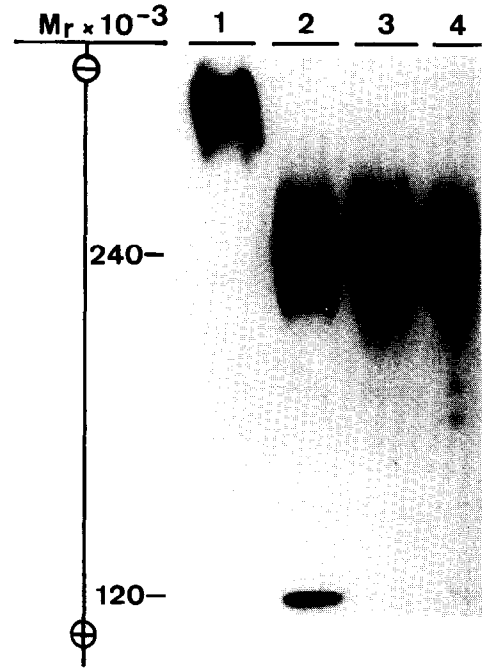

Fig.1. Polyacrylamide gel electrophoresis of the invertase produced by *Sc. pombe* 972 h⁻ (lane 1) and *S. cerevisiae* X2180-1A (lane 2) under derepressing conditions for invertase synthesis and by transformants of strains CY-239 (lane 3) and 972 h⁻ (lane 4) from *Sc. pombe* with plasmid pRB58, grown under repressing conditions. Numbers on the left-hand side of the gel are the molecular masses of large and small invertases from *S. cerevisiae*.

results were obtained when Endo H treatment was performed with extracts from cells grown under derepressing conditions for invertase synthesis (not shown).

3.3. *Synthesis of heterologous invertase under derepressing conditions*

Besides the *SUC2* structural gene for *S. cerevisiae* invertase, the pRB58 plasmid carries DNA from its 5′- and 3′-flanking regions; this permits the production of invertase by Suc⁻ mutants of *S. cerevisiae* transformed with the plasmid to be regulated by glucose [7]. To investigate whether the expression of the *SUC2* gene in *Sc. pombe* transformants was also regulated by catabolite repression, extracts from *Sc. pombe ura4-294* (pRB58) cells incubated for different times under derepressing conditions were subjected to electrophoresis and the gel was stained for inver-

Table 2

Precipitation of invertase produced by strain CY-239(pRB58) from *Sc. pombe* by Con A-Sepharose (A) and by the lectin from *Bandeiraea simplicifolia* seeds (B)

	Strain	Plasmid (pRB58)	Additions to invertase samples	Invertase activity	
				In supernatants (U)	Bound to lectin (%)
A	CY-239	+	–	0.66	–
		+	lectin	0.03	95
	972 h⁻	–	–	1.03	–
		–	lectin	0.03	97
	X2180-1A	–	–	0.92	–
		–	lectin	0.02	98
B	CY-239	+	–	0.42	–
		+	lectin	0.42	0
		+	galactose	0.44	–
		+	lectin + galactose	0.43	0
	972 h⁻	–	–	0.46	–
		–	lectin	0.04	91
	X2180-1A	–	–	0.40	–
		–	lectin	0.40	0

Invertases from strains *Sc. pombe* 972 h⁻ and *S. cerevisiae* X2180-1A were used as controls

tase activity. Fig.3 shows that whereas the amount of *Sc. pombe* genuine invertase progressively increased with the incubation time the heterologous enzyme seemed to be synthesized in a constitutive fashion, with no apparent increase in the activity as time progressed.

4. DISCUSSION

From the results offered in this paper it may be concluded that the heterologous gene *SUC2*, which codes for *S. cerevisiae* invertase, can be efficiently expressed after being introduced by transformation in *Sc. pombe* cells. The heterologous enzyme is glycosylated and secreted into the cell wall apparently behaving like the genuine invertase of *Sc. pombe*; however, the sugar moieties of both enzymes must differ in some aspects since the former is not bound by the lectin from *Bandeiraea simplicifolia* seeds, pointing to the absence of terminal galactosyl residues characteristic of *Sc. pombe* invertase [11].

The electrophoretic mobility of the heterologous enzyme and its sensitivity to Endo H indicate that

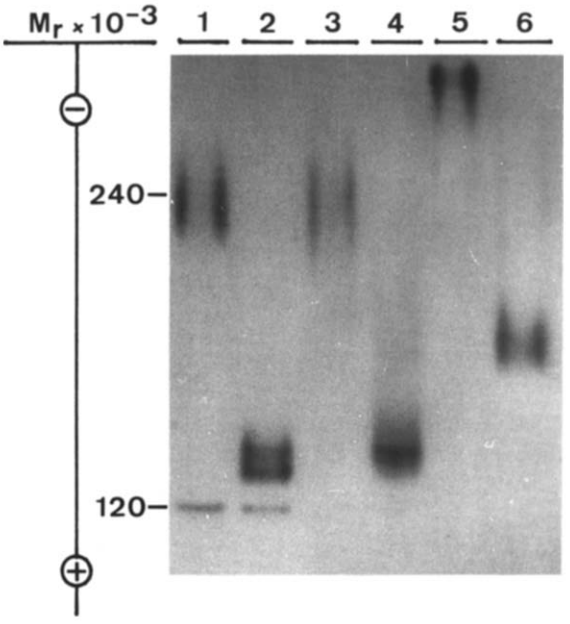

Fig.2. Polyacrylamide gel electrophoresis of the invertase produced by *S. cerevisiae* X2180-1A (1,2), *Sc. pombe* CY-239(pRB58) (3,4) and *Sc. pombe* 972 h⁻ (5,6) before (1,3,5) and after (2,4,6) treatment with Endo H under non-denaturing conditions. Numbers on the left-hand side of the gel indicate the molecular masses of large and small invertases from *S. cerevisiae*.

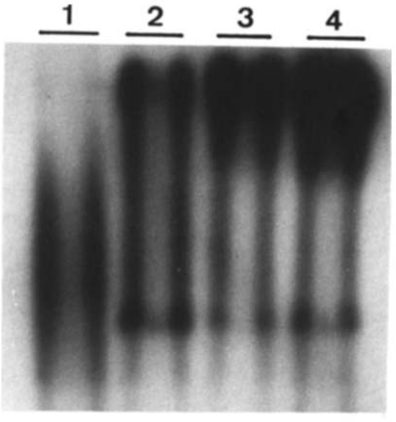

Fig.3. Polyacrylamide gel electrophoresis of the invertase produced by *Sc. pombe* 972 h⁻(pRB58) after 0 (1), 1 (2), 2 (3) and 6 (4) h of incubation under derepressing conditions for invertase synthesis. The four samples were prepared with approximately the same invertase activity.

the 9–10 out of 13 potential glycosylation sites which are *N*-glycosylated in *S. cerevisiae* invertase [9] are also *N*-glycosylated when the enzyme is expressed in *Sc. pombe*. Furthermore, the electrophoretic mobility of the deglycosylated heterologous enzyme, similar to that of the Endo H-treated *S. cerevisiae* invertase, and the fact that it is secreted can be interpreted as an indication that the signal sequence of *S. cerevisiae* invertase is functional in *Sc. pombe*, permitting the heterologous enzyme to insert itself into the ER membranes, the sequence being correctly cleaved in this step.

Altogether, the above results support the assumption that the signals for processing, glycosylation and secretion contained in *S. cerevisiae* invertase could be acknowledged by *Sc. pombe*, although, by contrast, the constitutive expression of the heterologous enzyme seems to indicate that the regulatory signals located along the *SUC2* gene in the pRB58 plasmid [7] are not recognized in the transformants.

The differences in the glycosylation patterns of the heterologous and genuine invertases synthesized by *Sc. pombe* could be attributed to a different spatial configuration of the proteins, which could modify the accessibilty of their glycosylation sites for some posttranslational-processing enzymes (i.e., galactosyltransferases); a similar hypothesis has been proposed to explain the host-dependent differences found in the glycosylation of several viral glycoproteins [23]. However, even if this were the case, it would also be a consequence of the primary structure of the protein moieties of the enzymes.

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