Essential role of Swp73p in the function of yeast Swi/Snf complex

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Swi/Snf protein was purified previously from the yeast Saccharomyces cerevisiae as an 11-polypeptide complex, including five novel Swp polypeptides. Here we present evidence concerning the role of Swp73p in the function of the complex. Deletion mutants in the SWP73 gene display phenotypes similar to those of swi and snf mutants, and in addition are temperature-sensitive. Swp73p is required for transcriptional activation by full-length glucocorticoid receptor (GR), but not by all GR derivatives. Swp73p is also required for activation with an enhancer element that binds the transcription factors Swi5p and Pho2p, which may underlie the defects in HO expression observed with swi and snf mutants. A single amino acid change in the protein confers phenotypes that are similar to those observed in the swp73Δ strain, but in some cases the two strains behave differently. The extent to which Swp73p is required for assisting transcriptional activation depends on the activator and promoter tested. Homologs of SWP73 are present in S. cerevisiae, Ashbya gossypii, Caenorhabditis elegans, and mice, indicating that SWP73 may belong to a family of related genes encoding proteins with analogous functions.

[Key Words: Swi/Snf complex; Saccharomyces cerevisiae; glucocorticoid receptor; transcription factors; HO; Swp73p]

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Packaging of DNA in nucleosomes represses transcription, and unpackaging, evidenced by nucleosome disruption, accompanies derepression (for review, see Winston and Carlson 1992). A number of proteins have been implicated in nucleosome disruption, including products of the yeast SWI/SNF genes and their counterparts in higher cells (Côté et al. 1994; Imbalzano et al. 1994; Kwon et al. 1994; for review, see Kingston et al. 1996). SWI/SNF genes were identified in genetic screens for mutants defective in the expression of the HO gene (which encodes an endonuclease required for mating-type switching; Stern et al. 1984), or mutants unable to ferment sucrose anaerobically (Neigborn and Carlson 1984; Breeden and Nasmyth 1987). Null mutations in any of these genes confer similar phenotypes, and all are suppressed by mutations in another set of genes, including those for histones, leading to the notion of a multi-protein complex opposing repression by chromatin (Kruger and Herskowitz 1991; Hirschhorn et al. 1992, Winston and Carlson 1992, Kruger et al. 1995). Isolation yielded a complex containing six SWI and SNF gene products and five additional polypeptides, termed Swp82p, Swp73p, Swp61p, Swp59p, and Swp29p (Cairns et al. 1994; Côté et al. 1994; Peterson et al. 1994). The complex was shown to perturb nucleosome structure in vitro, as judged from accessibility of the nucleosomal DNA to DNase I and to sequence-specific binding proteins (Côté et al. 1994).

The Swi/Snf complex apparently lacks specific DNA-binding activity, raising the question of how it is directed to specific promoters. A possible answer has come from studies of the rat glucocorticoid receptor (GR). A member of the intracellular receptor superfamily, GR enables hormone-dependent transcriptional activation of a minimal yeast promoter containing GR-binding sites [Schena and Yamamoto 1988]. Activation is potentiated by the Swi/Snf complex, and a physical association between GR derivatives and the Swi/Snf complex has been demonstrated [Yoshinaga et al. 1992]. Activation by GR in mammalian cells appears to involve a related human Swi/Snf complex and the perturbation of chromatin structure (Muchardt and Yaniv 1993).

Here, we describe two lines of work converging on the identification of Swp73p as a functionally important member of the yeast Swi/Snf complex. Pursuit of the SWP73 gene and its protein product revealed a role in transcriptional activation. Conversely, studies of the requirements for activation by GR in yeast led to the SWP73 gene and mutant derivatives.

Results

Identification of the SWP73 gene

To characterize members of the Swi/Snf complex, isola-
tion was performed by four ion-exchange steps followed by anti-Snf6p immunoaffinity chromatography as described in Cairns et al. (1994). The immunoaffinity column was subjected to stringent washing with 700 mM potassium acetate and 0.2% NP-40, and at least 10 polypeptides were eluted with 5 M urea, while the Snf6 protein remained bound to the column [Fig. 1A]. Immunoblot analysis identified five polypeptides in the eluate as Swi1/Adr6p, Swi2/Snf2p, Swi3p, Snf5p, and Tfg3/Anc1/Swp29p [Cairns et al. 1996]. A 20-kD polypeptide, encoded by the SNF11 gene, did not stain with silver, and has been observed only with staining with Coomassie blue [Treich et al. 1995]. In addition to these SWI/SNF gene products, four polypeptides were obtained, designated Swp59p, Swp61p, Swp73p, and Swp82p (Cairns et al. 1994).

For sequencing, Swi/Snf complex was resolved by SDS-PAGE and transferred to a synthetic membrane. The band attributable to Swp73p was excised and treated with trypsin, and the following two peptide sequences were obtained: LTDLLSINSLHILPLQFIEDYTVR and ASTYGELVLIDIEVPDVNLK. These sequences were a perfect match to residues 404–428 and 432–451, respectively, of the deduced amino acid sequence of the SCPC-2132 gene.

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from the peak fraction from Mono S (fraction no. 28, 4 μg), and either anti-Swp73p or anti-Tfg3p antibodies. Immune complexes were washed twice with buffer B containing 600 mM potassium acetate and 0.2% Nonidet P-40, then eluted with 5 M urea. The material half of the 5 M urea eluates of the washed anti-Swp73p or anti-Tfg3p immune complexes, respectively; (lanes 1, 2) half of the supernatants from the anti-Swp73p or anti-Tfg3p immunoprecipitations, respectively; (lanes 3, 7) half of the solublized pellet of the anti-Swp73p or anti-Tfg3p immune complexes. (D) Analysis of anti-Swp73p and anti-Tfg3p immune complexes by silver staining. The immune eluates (in 5 M urea, supplemented with SDS loading buffer) described in C were resolved in an SDS-8% polyacrylamide gel, and proteins were revealed by silver staining.

Swp73p component of Swi/Snf

from the peak fraction from Mono S (fraction no. 28, 4 μg), and either anti-Swp73p or anti-Tfg3p antibodies. Immune complexes were washed twice with buffer B containing 600 mM potassium acetate and 0.2% Nonidet P-40, then eluted with 5 M urea. The material remaining bound to the antibody beads, denoted pellet, was solublized by boiling the immune precipitates for 2 min in 2× SDS-PAGE gel-loading buffer. Samples were separated by SDS-PAGE in an SDS–10% acrylamide gel and immunoblotted. (Lanes 1, 5) Peak Mono S fraction (2 μg), (lanes 2, 6) half of the supernatants from the anti-Swp73p or anti-Tfg3p immunoprecipitations, respectively; (lanes 3, 7) half of the 5 M urea eluates of the washed anti-Swp73p or anti-Tfg3p immune complexes, respectively; (lanes 4, 8) half of the solublized pellet of the anti-Swp73p or anti-Tfg3p immune complexes. (D) Analysis of anti-Swp73p and anti-Tfg3p immune complexes by silver staining. The immune cluates (in 5 M urea, supplemented with SDS loading buffer) described in C were resolved in an SDS–8% polyacrylamide gel, and proteins were revealed by silver staining.

dehydrogenase (GPD) promoter. This strain was further transformed with pHCA–G3Z, a centromeric reporter plasmid containing three glucocorticoid response elements upstream of a minimal CYC1 promoter and the lacZ gene. The resulting strain [WG3Z7C] was mutagenized with ethane methylsulfonate; ~4800 survivors were treated with 10 μM deoxycorticosterone [an agonist of GR; Garabedian and Yamamoto 1992], and GR-dependent activation was assessed by determination of β-galactosidase activity in plate assays. Diminished levels of activity were quantitated in liquid assays. Thirteen mutants were isolated that produced β-galactosidase activity at levels at least fourfold below those displayed by the wild-type parent strain.

One of these 13 mutants [W525] displayed a similar defect with 1 μM deacylcortivazol, a second GR agonist [Garabedian and Yamamoto 1992], as well as a temperature-sensitive growth phenotype at 37°C (Ts−). Backcrossing W525 to the isogenic wild-type parent strain [W303-1B] and tetrad analysis of the resulting heterozygous diploid revealed a segregation pattern consistent with a mutation in a single gene and demonstrated linkage of the Ts− phenotype to the lack of GR activation.

Mutation in SWP73 responsible for defective GR activation

The mutated gene resulting in the GR defect in W525 was identified by complementation of the Ts− phenotype. W525 was transformed with a high-copy yeast genomic DNA library [LEU2-marked, 2 μ origin; Engelbracht et al. 1990] and 20,000 transformants were examined for growth at 37°C. A single colony was isolated that grew as well as the wild-type parent strain under these conditions, and that also exhibited β-galactosidase activity in liquid assays comparable to that observed with the parent strain. Complementation of both the Ts− phenotype and the defect in GR activation was linked to the plasmid (see Materials and Methods).

Isolation of the complementing plasmid, partial sequencing of the genomic library insert, and a search of the PIR protein sequence data base revealed that the insert contained the open reading frame of SWP73. To ensure that the complementation observed was not the result of an elevated dosage of Swp73p, the SWP73 gene was cloned in a centromeric plasmid [pUCA–SWP73]. Strain W525 harboring this plasmid did not display either the growth or GR activation defects observed with the same strain harboring a control plasmid, showing that the level of Swp73p produced from a centromeric plasmid is sufficient for complementation.

To establish further that a mutation in the SWP73 gene was responsible for the mutant phenotype of strain W525, the swp73 allele in W525 was mapped by gap repair with the use of pUCA–SWP73 [Rothstein 1991]. Recovery and sequencing of the gap-repaired plasmid
pUCA–SWP73(AR) revealed a single base pair mutation resulting in the substitution of aspartic acid for glycine at position 323. When the same substitution was brought about by site-directed mutagenesis of pUCA–SWP73, the resulting plasmid failed to support growth of W525 at 37°C and GR activity was diminished. Additional mutations substituting either lysine or leucine at amino acid position 323 behaved identically to G323D, indicating that these mutant phenotypes were most likely attributable to a change in the size of the amino acid residue at position 323 rather than a change in charge. With the mapping of this allele, denoted swp73-1, and the confirmation that a mutation in SWP73 was involved in the mutant phenotype of W525, the mutant strain was renamed Wswp73-1.

Phenotypes of swp73Δ strains are similar to those displayed by Wswp73-1, but of greater penetrance

Deletion of chromosomal SWP73 was brought about by a single-step gene replacement with a swp73::LEU2 disruption plasmid in the diploid strain W303 (designated W303-73Δ; see Table 5). Tetrad analysis of the resulting heterozygous diploid revealed that SWP73 is not an essential gene. In each tetrad tested, however, the two swp73Δ::LEU2 spores displayed a Ts− growth phenotype at 37°C and showed little or no GR activation (Fig. 3). The swp73Δ::LEU2 haploid strain [designated Wswp73Δ] derived from W303-73Δ also had a longer generation time than did W303-1A at 24°C (4.3 hr and 2.0 hr for the two strains, respectively) and at 30°C (5.3 hr and 1.6 hr). The Ts− phenotype was not limited to W303-derived swp73 mutants, as Yswp73Δ (YPH499 parent, S288C background) was also Ts−.

Wswp73-1 cells displayed an intermediate phenotype that resembled the deletion mutant [Wswp73Δ] more than the wild-type strain (W303-1A), requiring seven days to form small colonies at 37°C. At 24°C and 30°C, however, Wswp73-1 closely resembled W303-1A (similar generation times). The greater growth capacity of Wswp73-1 cells at intermediate temperatures may indicate that swp73-1p is partially functional in these cells. Immunoblot analysis, however, revealed that the level of swp73-1p in Wswp73-1 cells was about tenfold less than that of wild-type protein in Wswp73-1 cells harboring pUCA–SWP73 or in W303-1A [data not shown], raising the possibility that a reduced amount of protein might account for the mutant phenotype.

To test whether the phenotypic difference between Wswp73-1 and wild-type was attributable entirely to the single amino acid substitution in the swp73-1 allele, Wswp73Δ was transformed with pUCA–SWP73 and with one of three mutant derivatives [G323D, G323K, G323L], and growth was examined at 37°C (Fig. 4). Each of the plasmids bearing site-directed mutations in SWP73, as well as the plasmid recovered by allele rescue from Wswp73-1, was able to complement partially the Ts− phenotype of Wswp73Δ, resulting in behavior like that of Wswp73-1, including similar growth at 24°C and 30°C [data not shown]. Wswp73Δ harboring the mutant plasmids grew somewhat better than Wswp73-1 at 37°C but not nearly as well as Wswp73Δ harboring pUCA–SWP73 or the wild-type strain. Immunoblot analyses showed that the three mutant proteins were present at levels approximately two- to threefold higher than that of Wswp73-1p in Wswp73-1 cells [presumably because centromeric plasmids are typically present at one to three copies per cell]. Therefore, phenotypes of Wswp73-1 may be attributed to a combination of reduced protein level and diminished functional capacity of swp73-1p.

swp73Δ strains display a Snf− phenotype

Deletions in previously identified members of the Swi/Snf complex (swi1Δ, swi2Δ, swi3Δ, swi5Δ, swi6Δ) cause defects in growth on sucrose or raffinose under anaerobic conditions (Snf−). To investigate whether deletion of the SWP73 gene has similar consequences, swp73Δ strains were grown on rich medium containing sucrose or raffinose and amitomycin A, an inhibitor of electron transport, whose presence simulates anaerobic conditions (Neigeborn et al. 1986). Yswp73Δ and Wswp73Δ required 5 or 7 days to form colonies, respec-
harbor any plasmids. The plasmid pRS316 was the parent vector.

**Figure 4.** Growth ability of strains at 37°C. [A] Growth ability on YEPD at 37°C. [B] Schematic diagram illustrating which strains were tested. The strains tested are indicated with an arrow. The black arc surrounds W303-1A, the dark gray arc surrounds Wswp73-1, and the light gray arc surrounds Wswp73A. The plasmids that they harbored are indicated inside the diagram, except for W303-1A and Wswp73-1, which did not harbor any plasmids. The plasmid pRS316 was the parent vector for the other plasmids tested and acted as a control vector in this experiment.

tively, compared to 2 days for isogenic wild-type strains. The Wswp73-1 strain displayed an intermediate phenotype, resembling the wild-type parent more than the deletion strain, requiring 3 to 4 days to form colonies.

Because diminished levels of invertase (product of the *SUC2* gene, whose expression depends on the Swi/Snf complex) may account for the Snf- phenotype in other *swi/snf* mutants, invertase assays were performed on wild-type (W303-1A), Wswp73-1, and Wswp73Δ strains. Although the invertase level was only reduced approximately twofold in the Wswp73-1 strain, the Wswp73Δ strain lacked appreciable invertase activity. We conclude that *swp73* mutants, like other *swi/snf* mutants, display a Snf- phenotype related to the production of invertase.

**LexA–Swi/Snf fusion proteins require Swp73p to activate transcription, and LexA–Swp73p requires Swi/Snf**

Fusion of the DNA-binding domain (amino acids 1–87) of the bacterial repressor LexA to Swi2/Snf2p, Snf5p, Snf6p, Snf11p, Gal4p, or the activation domain of the rat GR (amino acids 1–452) creates fusion proteins that activate transcription of a promoter under control of a single *lexA* operator (Hanes and Brent 1989; Laurent et al. 1990, 1991; Laurent and Carlson 1992; Yoshinaga et al. 1992, Treich et al. 1995). The fusions to members of the Swi/Snf complex generally require the other complex members for full activation (Laurent and Carlson 1992). This approach is thought to bypass the requirement for an activator to target the Swi/Snf complex to a promoter, and provides evidence for function of Swi/Snf proteins in a complex that includes the other members. This approach was extended to our Wswp73Δ and Wswp73-1 strains, with the use of a β-galactosidase reporter plasmid containing a *lexA* operator upstream of a minimal

| Strain                      | Secreted invertase activity (µmole glucose/min per mg) | Derepressed |
|----------------------------|--------------------------------------------------------|-------------|
| W303-1A (wild type)        | 40                                                     | 2 hr        | 3 hr        |
| Wswp73-1                   | 13                                                     | 274         | 556         |
| Wswp73Δ                    | 21                                                     | 44          | 55          |

**GAL1** promoter. LexA–Snf2p and LexA–Snf5p fusion proteins, potent activators in the wild-type strain W303-1A, were ineffective in either the Wswp73Δ or Wswp73-1 backgrounds (Table 2). Transcriptional activation by LexA–Snf6p was reduced two- or tenfold in Wswp73Δ or Wswp73-1 strains, respectively. We conclude that *swp73* is required for transcriptional activation involving other members of the Swi/Snf complex.

Conversely, to determine whether other members of the Swi/Snf complex are required for function of Swp73p, a LexA–Swp73p fusion was tested in various *swi/snf* mutant backgrounds. This fusion protein proved to be a strong activator, driving production of 904 units of β-galactosidase activity in wild-type cells. In contrast, the fusion protein caused production of only 5 or 25 units of activity in *snf5Δ* (S288C background) and *Wsnp2Δ* (W303 background) strains, respectively. The fusion protein could also substitute for wild-type Swp73p, as expression of LexA–Swp73 in either Wswp73Δ or Wswp73-1 strains conferred full *Ts*+ and Snf- phenotypes. These findings indicate that Swp73p is a full functional member of the Swi/Snf complex.

**Certain activators that require Swi2/Snf2p also require Swp73p**

As mentioned above, *SWI2/SNF2* was originally identified as important for expression of both *SUC2* and *HO* genes. *HO* expression further involves the DNA-binding transcription factors *Swi5p* and *Pho2p* (also known as *Grl10p*), which function as a heterodimer. A 46-bp region from the *HO* promoter, which constitutes a heterodimer-binding site, enables *swi5/SPho2-dependent* transcriptional activation when placed in front of the *CYC1* promoter (Brazas and Stillman 1993). We found that transcriptional activation with the *Swi5p/Pho2p*-binding element was dependent on *Swi2/Snf2* and on *Swp73* as well, as only 6 or 13 units of β-galactosidase activity were obtained with *Wsnp2Δ* or *Wswp73Δ* strains, respectively, compared with 118 units of activity with the wild-type W303-1A strain. Wswp73-1 displayed an intermediate phenotype, yielding 42 units of activity. A requirement for *Swi/Snf* complex to mediate activation by *Swi5p/Pho2p*, therefore, may underlie the defect in *HO* expression observed with *swi2/snf2* mutants.

Swi2/Snf2p is reported to potentiate transcriptional
Table 2. Activation by LexA-activator fusion proteins assessed with one LexA operator and the GAL1 promoter

| Activator expressed | β-galactosidase activity (units/mg) |
|---------------------|-----------------------------------|
|                     | W303-1A | Wswp73Δ | Wswp73-1 | Wsnf2Δ |
| LexA-1-87 (control) | 10      | 8       | ND       | ND     |
| LexA-Snf2p          | 3787    | 49      | 55       | 5066   |
| LexA-Snf5p          | 3295    | 10      | 21       | ND     |
| LexA-Snf6p          | 13584   | 5396    | 1306     | 456    |
| LexA-Swp73p         | 904     | 708     | ND       | 25     |
| LexA-Gal4p          | 2691    | 2317    | 3784     | 141    |
| LexA-GR(1-452)      | 1185    | 1129    | 27       | 16     |

The indicated LexA-fusion proteins were expressed, and β-galactosidase assays performed as described in the Materials and Methods. Transformants also contained the reporter pLR1840, which contains one consensus binding site for LexA upstream of the GAL1 promoter fused to the β-galactosidase gene. The values reported are the average of at least four transformants done in duplicate. Standard errors were <20%. ND: not determined.

An activator that does not require Swi2/Snf2p also does not require Swp73p

Activation of PHO5 transcription by Pho4p is not dependent on Swi2/Snf2p (Schneider 1995). To test dependence on Swp73p, activity of the endogenous PHO5 promoter was assessed in Wsnf2Δ, Wswp73Δ, Wswp73-1, and wild-type [W303-1A] strains. Growth on low phosphate medium to induce PHO5 transcription and liquid assays of the PHO5 gene product acid phosphatase gave 496 units for strain Wsnf2Δ, 512 for Wswp73Δ, 206 for Wswp73-1, and 542 for W303-1A [wild type]. We assume that the twofold lower activity for Wswp73-1 is not significant, and therefore, Swp73p, like Swi2p/Snf2p, is not required for Pho4p activation of the PHO5 promoter.

Certain activators that require Swi2/Snf2p do not require Swp73p

Activation by LexA–Gal4p requires Swi2/Snf2p, Snf5p, and Snf6p when assessed with a reporter plasmid containing a minimal GAL1 promoter (and one lexA operator), but is independent of these Swi/Snf components when assessed with a minimal CYC1 promoter (Laurent and Carlson 1992). LexA–GR [amino acids 1–452 of GR fused to the LexA DNA-binding domain] behaved similarly to LexA–Gal4p with a lexA–GAL1 promoter, activating transcription in a wild-type strain but not in the isogenic Wsnf2Δ strain (Table 2). In contrast with the

Table 3. Activation by various transcriptional activators assessed with a CYC1 promoter reporter plasmid

| Activator expressed      | Reporter plasmid | β-galactosidase activity (units) |
|--------------------------|------------------|---------------------------------|
|                          |                  | W303-1A | Wswp73Δ | Wswp73-1 | Wsnf2Δ |
| Full-length GR (N795)    | pHCA-G3Z         | 824     | 86      | 84       | 9      |
| Constitutive GR (N556)a  | pHCA-G3Z         | 1167    | 921     | 125      | 20     |
| Constitutive GR (N556)b  | pAS26x           | 2716    | 1150    | 180      | ND     |
| Yap1p (endogenous)      | pAS-TRE2         | 996     | 79      | 951      | 124    |
| RARβ                    | pAS-DR5          | 303     | 24      | 5        | ND     |
| Gal4p (endogenous)      | pSV14            | 4794    | 1029    | 589      | ND     |
| Gal4p (endogenous)      | p121-Δ10         | 1577    | 24      | <1       | ND     |

The indicated activators were expressed, and β-galactosidase assays performed. See Materials and Methods for the identity of reporter plasmids. The values reported are the average of at least four transformants done in duplicate. Standard errors were <20%. ND: not determined.

aCentromeric expression plasmid pTCA-GN556.
b2µ origin expression plasmid pGN556.
requirement for Swi2/Snf2p, Swp73p was dispensable, as the levels of transcription observed in the Wswp73Δ strain were indistinguishable from those in the wild-type strain. The lack of Swp73p dependence was not promoter specific, as shown by experiments with a reporter plasmid containing the CYC1 promoter (and one lexA operator). Activation by LexA-GR or LexA-Gal4p was reduced only two- or threefold, respectively, in Wswp73Δ cells compared with wild-type cells [Table 4]. These findings raise the possibility that Swp73p performs roles in the Swi/Snf complex distinct from those of Swi2/Snf2p.

Activator dependence of swp73-1p function

As already mentioned, Wswp73-1 cells behave more like Wswp73Δ cells than wild-type in regard to the Ts− phenotype, whereas they resemble wild-type more closely in regard to the Snf− phenotype. Evidently some functions of the Swi/Snf complex are more affected by the swp73-1 mutation than others. In keeping with this variation, we observed differences in potency among activators in Wswp73-1 cells. LexA–Snf2p, LexA–Snf5p, full-length GR, and RARβ were ineffective in Wswp73-1 cells [as in Wswp73Δ cells], whereas LexA–Gal4p retained full activity [Table 2]. Other activators, such as a constitutive version of GR [N556], endogenous Gal4p [assessed with pSV14], and Swi5p/Pho2p showed an intermediate dependence. An unexpected finding was that two activators, LexA–GR [assessed with pLR1840] and Yap1p, were more effective in Wswp73Δ cells than in Wswp73-1 cells. This behavior did not reflect variation in abundance of the activators, as Wswp73Δ and Wswp73-1 transformants contained approximately the same amount of LexA–GR as did wild-type cells [data not shown]. The diminished effectiveness of LexA–GR in Wswp73-1 cells was promoter dependent, as little loss in activity was seen with the CYC1 promoter [Table 4]. Apparently, swp73-1p can either facilitate or interfere with transcriptional activation, depending on the cellular conditions and promoter context (see Discussion).

Table 4. Activity by LexA-GAL4 or LexA-GRact fusion proteins assessed with one LEXA operator and the CYC1 promoter

| Activator expressed | W303-1A | Wswp73-1 | Wswp73Δ |
|---------------------|---------|----------|---------|
| LexA–1–87 (control) | 27      | ND       | ND      |
| LexA–Gal4           | 11704   | 11222    | 3800    |
| LexA–GR[1–452]      | 10886   | 6722     | 5206    |

The indicated LexA-fusion proteins were expressed, and β-galactosidase assays were performed as described in the Materials and Methods. Transformants also contained the reporter pASLEX, which contains one consensus binding site for LexA upstream of the CYC1 promoter fused to the β-galactosidase gene. The values reported are the average of at least four transformants done in duplicate. Standard errors were <20%. ND: not determined.

Swp73p is significantly similar to proteins in yeast and higher eukaryotes

The protein sequence of Swp73p was compared to other proteins in the National Center for Biotechnology Instruction [NCBI] data base with the BLAST program, which identified significant similarity to the polypeptides encoded by one partial and three full open reading frames: AgTHR4gns (from Ashbya gossypii, a filamentous fungus), YCR052w (from Saccharomyces cerevisiae), D15Kz1 (from Mus musculus), and ZK1128.5 (from Caenorhabditis elegans; Johnston et al. 1989; Oliver et al. 1992; Altmann-Jöhl and Philippsen 1996; M. Burks, unpub.). As calculated by the program BESTFIT, Swp73p is 39% identical and 57% similar to AGTHR4gns protein over 223 amino acids, 34% identical and 57% similar to Ycr052p protein, 29% identical and 53% similar to D15Kz1 protein, and 26.5% identical and 53% similar to ZK1128.5 protein. At present only D15Kz1 has been described further, with preliminary results showing that the corresponding mRNA, although ubiquitous, is most highly expressed in the developing nervous system [Johnston et al. 1989].

Alignment of the four related protein sequences showed that the similarity among them extends throughout their length [Fig. 5]. The amino-terminal portion of the protein encoded by AgTHR4gns is not yet available, but the extent of homology with Swp73p in the carboxy-terminal region suggests that the two proteins are true homologs and that the carboxy-terminal portion is important for function. The two S. cerevisiae proteins, although similar in sequence, could not substitute for one another, as neither high copy nor centromeric plasmids bearing YCR052w could suppress the Ts− phenotype of either Wswp73-1 or Wswp73Δ (data not shown). Only the yeast protein Ycr052p contains a glycine residue at the position corresponding to that of the glycine to aspartic acid mutation in swp73-1p (position 323 in Swp73p). The two yeast proteins are more similar to one another than to the mouse or C. elegans proteins, but the similarity between the mouse and C. elegans proteins is also high (56% identical and 73% similar), raising the possibility that these proteins are functional homologs of Swp73p.

Discussion

Our findings demonstrate both a tight physical association of Swp73p with the yeast Swi/Snf complex and an important role of the protein in the function of the complex. The SWP73 gene was obtained independently by sequencing the purified protein and from a genetic screen for mutants defective in mediating transcriptional activation by GR. The swp73-1 mutant allele exhibited Ts− and Snf− phenotypes, in addition to defects in GR activity, and similar phenotypes were obtained by SWP73 deletion. All members of the Swi/Snf complex that are required for Snf+ and Swi+ phenotypes are also required for activation by GR [Yoshinaga et al. 1992], and we demonstrate a functional interdependence between
Figure 5. Swp73p is highly similar to four other polypeptides. Shown is the multiple sequence alignment of the predicted polypeptides encoded by SWP73, ATGTHR4GNS, YCR052w, D15Kz1, and ZK1128.5. Only a partial open reading frame has been determined for ATGTHR4GNS, therefore the predicted polypeptide that is presented is only the carboxy-terminal sequence. For the other sequences the translational start codons have not been determined experimentally, therefore we have assumed that the first AUG codon downstream of the TATA box is the initiator. Positions of absolute identity in all the proteins are indicated with a bullet. Positions at which all proteins have a similar amino acid are indicated with an asterisk. The glycine that was converted to an aspartic acid in swp73-1 is indicated with a downward arrow. Gaps are shown with dashes. Regions of absolute identity and high similarity among all the proteins are depicted with shaded boxes. Regions of identity and high similarity among only the yeast homologs are depicted with outline boxes. Note that the carboxy termini of Swp73p and AGTHR4GNS are almost identical (78% identity and 91% similarity over 45 amino acids).

Swp73p and other complex members. In all likelihood, Swp73p subserves its role in support of GR and other activators in the context of the Swi/Snf complex.

Although the behavior of Swp73p largely parallels that of previously documented members of the Swi/Snf complex, some distinctions were also noted. For example, activation by LexA–Gal4p and that by LexA–GR depend strongly on Swi2/Snf2p, yet neither requires Swp73p. In contrast endogenous Gal4p and full-length GR depend both on Swi2/Snf2p and Swp73p for activation. Whether these differential effects of Swp73p are a consequence of differential expression of the fusion and full-length proteins or differences in the activator molecules themselves has not yet been determined. In any case it is clear that Swp73p is relatively unimportant in certain conditions in which Swi2/Snf2p is strongly required.

The Ts− phenotype of swp73Δ strains has not been reported for other swi and snf mutants, and may represent an additional functional difference between Swp73p and other members of the Swi/Snf complex. We find that swi1Δ and swi3Δ strains (derived from the wild-type yeast strain S288C) are only slightly sensitive to elevated temperature, whereas a congenic swi2Δ strain also grows at 37°C, albeit slowly. Thus, Swp73p may perform a function at 37°C that is independent of the Swi/Snf complex. Swi/Snf subcomplexes have been reported in strains lacking certain components of the complex (Peterson et al. 1994), and Swp73p might be an obligate member of a subcomplex able to function at 37°C. The lesser penetrance of the Ts− phenotype in Wswp73-1 may be attributable to a lesser impairment of function by the mutation compared with deletion, or to instability of swp73-1p (present at ~10% of the level of Swp73p in wild-type cells).

Four classes of transcriptional activators may be defined on the basis of their behavior in Wswp73-1 and Wswp73Δ cells (Fig. 6): (1) those that were equally affected in both mutant strains; (2) those that were equally unaffected in both mutant strains; (3) those that were more negatively affected in Wswp73Δ and (4) those that were more negatively affected in Wswp73-1. Perhaps most notable are the class IV activators, which function normally in the swp73Δ strain but are severely defective in the swp73-1 background. One interpretation is that Swi/Snf complexes formed in the absence of Swp73p differ from those formed in the presence of swp73-1p. According to this idea, the complexes lacking Swp73p are as active with class IV activators as the wild-type Swi/
Figure 6. The transcriptional activators tested fall into four classes. Shown is a schematic diagram summarizing these results. Class I is defined as those activators and promoter elements that were equally negatively affected in Wswp73-1 and Wswp73Δ and is denoted as “Wswp73-1 = Wswp73Δ”. Class II is defined as those activators and promoter elements that were equally unaffected in the two strains and is denoted as “Wswp73-1 = Wswp73Δ”. Class III is defined as those activators and promoter elements that were less affected in Wswp73-1 and is denoted as “Wswp73-1 < Wswp73Δ”. Class IV is defined as those activators and promoter elements that were more affected in Wswp73-1 and is denoted as “Wswp73-1 > Wswp73Δ”. The activators in each case are listed in parentheses: (LexA-Snf2p, LexA-Snf5p, GR[N795], RARp, Gal4p*) (LexA-Gal4p, Pho4p, and LexA-GR*)

Class I: Wswp73-1 = Wswp73Δ

Class II: Wswp73-1 = Wswp73Δ

Class III: Wswp73-1 < Wswp73Δ

Class IV: Wswp73-1 > Wswp73Δ

Swp73p component of Swi/Snf

Snf complex, whereas the complex containing the mutant swp73-1p is inactive with these activators. In this cellular context, swp73-1p appears to be an inhibitor of Swi/Snf action and may reflect the formation of nonproductive complexes. In addition, the effects of the swp73-1 mutation and the SWP73 deletion differ in different promoter settings; for example, LexA–GR activates the lexA–CYC1 promoter equally in the two genetic backgrounds, but displays lower activity on the lexA–GAL1 promoter in swp73-1 than in swp73Δ (Tables 2 and 4). Thus, it appears that the phenotypic effects of SWP73 mutations are highly dependent on both activator and promoter contexts.

The different versions of GR that were tested were (Gal4p*)Gal4p assessed with pSV14; (Gal4p**)Gal4p assessed with pl21-A10; (LexA-GR*) LexA-GR assessed with pLR1840. Schematic diagrams illustrating the state of the Swi/Snf complex in W303-1A (left in each set), Wswp73-1 (middle in each set), and Wswp73Δ (right in each set) are included. In each class, the relative activity of the activators from their promoter elements in these strains is indicated by +, ++, +++|. Strong activation; ++| intermediate activation; + poor activation.
and BspEI and introduced into W303 by electroporation [Becker and Guarente 1991]. Correct integration was confirmed by Southern blot analysis. The Leu− phenotype cosegregated with the Ts+ phenotype and the Leu+ phenotype cosegregated with the Ts− phenotype in all tetrad analyzed. An additional strain lacking the SWP73 gene (Yswp73Δ) in the S288C genetic background was prepared in the same manner by a one-step gene replacement with pRL100 and the haploid parent strain YPH499. The swt1A strain CY58 was a gift of Craig Peterson (University of Massachusetts, Worcester), and is congenic to YPH499.

A haploid strain lacking the SNF2 gene, Wsnf2a [YBC402], was obtained from tetrad dissection of the heterozygous snf2Δ/ snf2Δ strain YBC402, which was prepared by transformation of W303 with BamHI-digested pKOSNF2L. Replacement of one of the SNF2 loci with the LEU2 gene by γ-transformation (Sikorski and Hieter 1989) was confirmed by Southern blot analysis. Sporulation yielded tetrads with two wild-type and two slow-growing spores. In all eight four-spore tetrads tested, the slow growth phenotype segregated with Snf− and Leu+ phenotypes. To characterize further the isolated haploid strain W303-1A, which was obtained from tetrad dissection of the heterozygous W303-1A/ W303-1B strain background (Thomas and Rothstein 1989), the snf2Δ:LEU2 strain W303-73Δ was transformed with YCP50-SNF2. All eight transformants tested displayed Snf+ phenotypes and grew as well as wild-type cells.

**Plasmids**

Expression plasmids for LexA fusion proteins, derivatives of pSH2-1 [HIS3, 2μ origin, expresses LexA [1–87] from the ADH1 promoter, gift of R. Brent [Harvard Medical School, Boston, MA]], were as follows: pLexA-Snf2, pLexA-Snf5, and pLexA-Swp73 [Laurent et al. 1990, 1991] gifts of B. Laurent; pLexA-Gal4 [pSH17-4], gift of R. Brent [Hanes and Brent 1989]; and pG-NLX [TRP1, 2μ origin, expresses LexA (1–87) from the GPD promoter, Yoshinaga et al. 1992]. For preparation of pLexA– SWP73.6XHIS, which expresses LexA [1–87] fused to full-length Swp73p with six histidine residues at the carboxyl terminus, a 1.8-kb DNA was amplified from genomic DNA by polymerase chain reaction (PCR) with the following oligonucleotide primers: 5′-CCCGAATTCACCATGGCCAAAGTAATGAAAC-CCAGCAAC-3′ and 5′-CCCAGATCTCTAATGGTGATGGTGCATTCGTCCATTTGATAATATGAC-3′. The PCR product was digested with EcoRI and BglII and inserted between the EcoRI and BamHI sites of pSH2-1.

For preparation of pET11D–SWP73.6XHIS, which expresses full-length Swp73p with six histidine residues at the carboxyl terminus, the 1.8-kb PCR product described above was digested with Ncol and BglII and inserted between the Ncol and BamHI sites of pET11D [Novagen, Inc.].

The GR expression plasmids pGN795 and pGN556, and the 3GRE–lacZ reporter plasmid pAS26x were as described [Yoshinaga et al. 1992]. The GR expression plasmid pTCA–GN795 consists of the sequence from pGN795 between the HindIII and NcoI sites, which contains the GDP promoter, the full-length GR coding sequence, and the phosphoglycerolkinase [PGK] terminator sequence, inserted between the same sites of pRS314 [TRP1, CEN4] [Sikorski and Hieter 1989]. Construction of pTCA–GN556 was accomplished in the same way except that pGN556 was used as the source of the HindIII–NcoI DNA fragment. The GR reporter pHCA–G3Z was constructed by moving the 3GRE–lacZ reporter fragment from pAS26x into pRS313 [HIS3, CEN4] [Sikorski and Hieter 1989]. The RARβ expression and reporter plasmids pG–RARβ and pΔS–ΔS5 were as described [Chen et al. 1993].

The Yap1p reporter pUCAS.TRE2 was constructed by inserting the Sall–BamHI fragment of pSM38 [Moye-Rowley et al. 1989], containing two SV40 AP-1 recognition elements and a minimal CYCI promoter between the Xhol and BamHI sites of pUCASS [Picard et al. 1990]. The Gal4p reporter p121Δ10 (West

### Table 5. Yeast strains

| Strain  | Relevant genotype                         | Source                                      |
|---------|-------------------------------------------|---------------------------------------------|
| W303    | tMATα/a                                   | A. Tzagoloff [Columbia University, New York, NY] |
| W303-1A | MATα                                      | A. Tzagoloff                                |
| W303-1B | MATα                                      | A. Tzagoloff                                |
| W303-73Δ | MATα/a                                  | This study                                 |
| W307C7Δ | MATα                                      | This study                                 |
| W3077-1C | MATα                                      | This study                                 |
| W3077Δ | MATα                                      | This study                                 |
| W3072ΔC | MATα                                      | This study                                 |
| MCY2064 | MATα                                      | B. Laurent [State University of New York, Brooklyn] |
| YPH499  | MATα                                      | P. Hieter [Johns Hopkins School of Medicine, Bethesda, MD] |
| Yswp73Δ | MATα                                      | This study                                 |

**Footnotes:**

- **W303** strain background [Thomas and Rothstein 1989].
- **W303-1A** strain background.
- **Derived from tetrad dissection of a W303 derivative.
- **YPH499** background.
contains a single, near-consensus, synthetic 17-mer Gal4p-binding site upstream of CYC1-lacZ. The Swi5p/Pho2 reporter plasmid was HO46-CYC1-lacZ [M632] (Brasza and Stillman 1993). For preparation of pUC4--SWP73, the region of the genomic clone of SWP73 from the SaI site in the upstream promoter (located at nucleotide position −219) to the XbaI site in the 3′ untranslated region (located 50 nucleotides downstream of the stop codon) was inserted into the XhoI and XbaI sites of pRS316 [URA3, CEN4; Sikorski and Heiter 1989].

The SNF2 disruption plasmid pKOSNF2L, designed to replace 92% of the SNF2-coding region with the LEU2 gene, was prepared from pRS305 and two PCR fragments of the SNF2 gene. The first fragment contained 600 bp of the 5′-noncoding region from nucleotides 220−820 [SNF2 initiator codon starts at nucleotide 890], except that the SacI site at nucleotide 228 was destroyed. This fragment was prepared from plasmid YCP50-SNF2 and the following oligonucleotide primers: 5′-CGCCGGATCCATGGCGCTGACCTTCCCGATGACAGAATAGCTGACCTTCCCGATGATCGCCGAGCTCGAG-3′. The second PCR fragment contained 722 bp of the 3′ coding and noncoding region from nucleotides 5288−6000 (termination codon starts at position 5587), and was prepared with the following oligonucleotide primers: 5′-CGCCGCCGCGCCAGAATTACATCAACAGACATAGCCACCAGCCCTTGAATCAAGTCCA-3′ and 5′-GCGCCGCCGCGCCAGAATTACATCAACACGACTTCCCGATG-3′. The first PCR fragment was digested with SacI and BamHI, the second fragment was digested with PsI and BamHI, and pRS305 was digested with PsI and SacI. A three-piece ligation with the digestion products yielded pKOSNF2L.

Purification of Swi/Snf complex

Two strains, BJ926 and Fleischmann’s active yeast (gift of Fleischmann’s Yeast Inc., Oakland, CA), were used in a purification procedure previously described (Cairns et al. 1996). Briefly, extract was prepared from 12 kg of cells and three chromatographic steps (Bio-Rex 70, DEAE–Sephacel, and hydroxylapatite) were performed according to Sayre et al. (1992). Peak hydroxylapatite fractions were further resolved on Mono Q, diluted twofold in buffer A [20 mM Tris-acetate (pH 7.6), 20% glycerol, 1 mM dithiothreitol, 1 mM EDTA, 2 μg/ml of chymostatin, 2 μg/ml pepstatin A, 0.6 μg/ml leupeptin, 2 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, and 0.01% Nonidet P-40], but lacking lithium chloride. The resulting slurry was centrifuged at 12,000Xg at 4°C for 15 min, and the pellet was resuspended in 25 μl of buffer C containing 1 μM lithium chloride, and 2% Nonidet P-40, followed by brief sonication. The round of centrifugation, resuspension, and sonication was repeated, and the material was subjected to two additional rounds with buffer C containing 2% Nonidet P-40, but lacking lithium chloride. The resulting pellet contained 80% pure Swi3p, as judged by SDS-PAGE and staining with Coomassie blue.

Antibodies

Purified recombinant Swp73p [5 mg] was subjected to electrophoresis in an SDS–10% acrylamide gel, stained with Coomassie blue dye. The Swp73p band was excised, and 0.5 mg was used to immunize rabbits, followed by boosting every 2 weeks with 0.25 mg. Polyclonal antiserum to Snf6p was prepared as described previously (Cairns et al. 1994). Affinity-purified Tfg3p antiserum was prepared as described (Henry et al. 1994). Polyclonal antiserum to Swi3p was a gift of Craig Peterson.

Immunoprecipitation

Either crude or affinity-purified Swp73p antiserum was coupled to protein A–Sepharose as described (Cairns et al. 1994). For preparation of anti-Tfg3p and anti-Swp73p immune complexes, the peak Swi/Snf fraction from the Mono Q column [4 μg] was diluted twofold in buffer A lacking potassium acetate and pre-cleared with 20 μl of 50% protein A–Sepharose beads [in buffer A containing 0.1 μM potassium acetate]. Samples were sedimented in a microcentrifuge and the supernatants were incubated with either 20 μl of 50% protein A–Sepharose beads coupled to anti-Tfg3p or 20 μl of 50% protein A–Sepharose beads coupled to anti-Swp73p antibodies for 3 hr at 4°C. The beads were sedimented in a microcentrifuge, and the resulting "IP"
Allele mapping and recovery of Wswp73-1

Linear DNAs resulting from cleavage of pUCA–SWP73 with BamHI and Clal or with Clal and HindIII were transformed into Wsp73-1 and uracil prototrophs were selected by growth on SD–ura plates. Only four of eight transformants from Clal–HindIII digested DNA displayed a temperature-sensitive growth defect (Ts-), whereas all eight transformants from Clal–HindIII digested DNA were Ts+. This result indicated that the mutation in swp73-1 lay between the Clal and HindIII sites. The following primers were synthesized to sequence the region between the Clal and HindIII sites in the gap-repaired plasmid pUCA–SWP73; 5′-GGTCGATTATTGGACAT-3′ (SWP73-1), 5′-CAGCACCCCAATTTGAC-3′ (SWP73-2), 5′-GTTGAATGGCAGATATGCCGC-3′ (SWP73-5). Sequencing revealed a single guanine to adenine change at nucleotide position 988 of the SWP73 open reading frame that results in a glycine codon to aspartic acid codon change at amino acid position 323.

DNA sequencing

Double-stranded plasmid templates were prepared and sequenced using the Sequenase 2.0 kit (U.S. Biochemical). The sequencing reactions were performed according to Del Sal et al. [1989]. Terminated fragments were labeled by incorporation of [α-32P]dATP (New England Nuclear), resolved in 6% acrylamide gels, and autoradiographed on Kodak X-Omat AR film. Sequencing of the genomic SWP73 clone was performed using primers that hybridize to the polylinker of YEp351. Sequences internal to the 4.5-kb genomic insert were determined by making a deletion of the sequence between the HindIII site of SWP73 and the HindIII site in the polylinker of the genomic clone. This deletion allowed the use of the downstream primer to sequence the middle of the insert. In addition to the sequence of the upstream promoter of SWP73 that is found in GenBank, the following sequence is found just upstream: 5′-GTCGACCT-GTTTCTG-3′.

Site-directed mutagenesis of SWP73

The following primers were synthesized and used to mutagenize pUCA–SWP73: 5′-GGAAAATATTGCATCATGTGAT-3′ (SWP73-A), 5′-GGAAAATATTGCATCATGTGAT-3′ (SWP73-L). Mutagenesis was performed using the Chameleon Double-Stranded, Site-Directed Mutagenesis kit (Stratagene) according to the manufacturer's instructions. DNA sequencing, using the SWP73-5 primer, confirmed the presence of the mutation.

Enzyme assays

Transcriptional activation by LexA fusion proteins in vivo was quantified with β-galactosidase assays using either the reporter plasmid pLRt840 (gift of R. Brent) or pASLex (gift of B. Laurent). Each of which contains a single high-affinity binding site for the Escherichia coli repressor LexA. Activation by SwisP/Pho2p was assayed in vivo using the reporter plasmid pHO–CYC1–lacZ [M632] (Brazas and Stillman 1993). Strains containing a reporter plasmid alone or in combination with plasmids expressing LexA fusion proteins were grown in 100 ml of synthetic medium containing the appropriate amino acids and 2% glucose to an optical density at 600 nm of 0.5–0.8. The cell suspension was centrifuged at 5000g for 10 min, washed with sterile water, harvested, and suspended in 0.5 ml of buffer A containing 400 mM sodium chloride. The cells were disrupted by beating with glass beads for 9 min at 4°C. The extract was centrifuged at 13,000g for 10 min, and the supernatant was centrifuged again at 13,000g for 10 min. β-Galactosidase activities, determined as described by Miller (1972), are given in units per microgram of protein in the cell extract.

Transcriptional activation by all other proteins was quantified by liquid β-galactosidase assays as described (Garabedian 1993), except that hormone treatment (when indicated) was for 6 hr, and the addition of SDS was eliminated from the permeabilization step.

Invertase assays were performed according to the method of Goldstein and Lampen (1975), as modified by Celenza and Carlson [1984]. Liquid acid phosphatase assays were performed as described (O’Neill et al. 1996), except that 1 mM sodium carbonate was used to stop the reaction. Acid phosphatase units are defined as 1000[A400/A290 V t-1], where t = time [min] and v = the cell fraction used [t = 10 and v = 0.1 for these experiments].

Sequence analyses

Homologs of Swp73p were identified with the program BLAST at the NCBI [Altschul et al. 1990]. The following parameters were used: database, nonredundant; expect, 10; cutoff, by default calculate using the expect values; matrix, blosum 62; word length, 3. Multiple sequence alignment was performed with a combination of the program PILEUP (Genetics Computer Group, Madison, WI), the alignments derived from the BLAST output, and visual inspection. The BESTFIT program (Genetics Computer Group) parameters used to determine percent identity and percent similarity were as follows: weight gap, 2.0; gap length 0.1; scoring matrix, swgappep.cmp.

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Note: The accession numbers for each of the putative SWP73 homologs described in this paper are: SWP73 (European Molec-
ular Biology Laboratory [EMBL] X62430, PIR S19063], AgTHR4gns [EMBL X91046], YCR052w [EMBL X59720, PIR S19466], D15K21 [GenBank M25773, PIR A30222], ZK1128.5 [EMBL Z47357].

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Note added in proof

Mutations in SIN1/SPT2 will suppress the Snf− and Swi− phenotypes of swi and snf mutants [Kruger et al. 1995]. To show further that the Ts phenotype of W303-1A is related to a defect in Swi/Snf function, we prepared a swp73Δ sin1/spt2Δ strain. The sin1/spt2Δ mutation suppressed the Ts defect conferred by swp73Δ, restoring a rate of growth resembling wild-type W303-1A at 37°C on solid rich media.

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