J. Biochem. 132, 557–563 (2002)

Functional Mapping of *Saccharomyces cerevisiae* Prp45 Identifies the SNW Domain as Essential for Viability[1]

Katerina Martinkova,[2] Pavel Lebduska,[2] Michal Skruzny, Petr Folk, and Frantisek Puta[3]

Department of Physiology and Developmental Biology, Charles University, Vinicna 7, 128 00 Praha 2, Czech Republic

Received May 3, 2002; accepted July 22, 2002

The essential gene product Prp45 (379 aa) of *Saccharomyces cerevisiae* is a highly conserved, but N-terminally abridged, ortholog of the human transcriptional coactivator SKIP, which is involved in TGFβ, Notch, and steroid hormone signaling. We used a diploid strain harboring *PRP45* deletion, which is inviable in the haploid, to test for complementation with the truncated versions of Prp45. The N-terminal half of the protein (aa 1 to 190), denoted as the SNW domain, was found sufficient to support the essential function. Interestingly, substituting the SNW motif itself with AAA was compatible with viability. GFP-tagged Prp45 was localized in nuclear "speckles" over a diffuse nuclear background. We further found that Prp45 activated the transcription of a reporter gene in *S. cerevisiae* when targeted to DNA. The observed effect relied in part upon the presence of conserved helical repeats and upon the highly charged C-terminal domain (pI = 11.3). Prp45, which lacks most of the binding motifs of the human ortholog, and whose N-terminal half is sufficient for supporting the growth of *prp45* cells, might be helpful in elucidating the essential function of SNW/SKIP proteins.

Key words: Bx42, complementation, DNA targeting, FUN20, SKIP.

Signal transduction networks controlling the transcription by RNA polymerase II are intimately connected with the regulation of chromatin structure and accompanying processes, such as pre-mRNA splicing and export (*1*). The downstream partners of transcription factors, transcriptional co-regulators, play a prominent role in this task, modifying nucleosomes (*2*), targeting nucleoskeletal components (*3*), or interacting with splicing complexes (*4*).

Prp45 is an ortholog of the human transcriptional coactivator SKIP, and apparently represents an indispensable regulatory element in eukaryotic cells. It was identified during the analysis of *Saccharomyces cerevisiae* chromosome I as an essential gene with an unknown function (FUN20; Function UNknown 20) (*5*). The authors were unable to render the gene product temperature-sensitive by random mutagenesis (*6*). Two-hybrid data from systematic screens in *S. cerevisiae* (*7, 8*), as well as preliminary information in a Saccharomyces Genome Database (*9*), suggest the participation of Prp45 in pre-mRNA processing.

The findings that human SKIP binds and potentiates the activity of the vitamin D receptor (VDR) or other steroid receptors (*10*) make it fit the role of a transcriptional coactivator. SKIP also augments TGFβ-dependent transcription on some Smad2/3 dependent targets (*11*). It was found to interact with these stimulatory Smad factors and with the inhibitory oncoprotein Ski (*12, 13*). SKIP was also proved to bind the downstream transcription factor CBF1 (RBP-Jκ) and several of the components of the hypothetical CBF1-containing DNA-complex, namely CIR, HDAC2, Sin3A, SMRT, and the transcription-activating fragment of the transmembrane receptor Notch (Notch IC) (*14, 15*). As in the previous case, binding was shown to both the stimulatory as well as to the inhibitory elements of the signaling cascade.

Independent observations of SKIP or its ortholog, Snw1p, from *Schizosaccharomyces pombe* indicated that it is associates with spliceosomal snRNPs (*16*), localizes to interchromatin granule clusters (*17*), or binds to the auxiliary splicing factor U2AF35 (*18*) suggesting a liaison between transcription initiation and the accompanying nuclear events, such as pre-mRNA processing. The presence of the gene in primeval eukaryotes and its essential character in both yeasts (*18*) argues for a very ancestral function. It should be interesting to find out the nature of this function and how its regulation is implemented by a wide range of transcription factors in higher eukaryotes.

We found that both Snw1p of *S. pombe* and SnwA of *D. discoideum* activate the transcription of a reporter gene in budding yeast when specifically targeted to its promoter (unpublished). Such behavior is seen with transcriptional coactivators, *e.g.* histone acetyltransferases (*19*), and many other proteins that partake in the regulation of transcription (*20*). We asked if the autologous Prp45 would also activate transcription when fused to a DNA-binding domain, and what parts of it would be indispensable for the essential function.

[1] This work was supported in part by grants Nos. 261/1999 of Charles University, 113100003 of the Ministry of Education and 204/02/1512 of the Grant Agency of the Czech Republic.

[2] These authors contributed equally to the work.

[3] To whom correspondence should be addressed. Tel: +420-2-2195-3147, Fax: +420-2-2195-3243, E-mail: dicty@mbox.cesnet.cz

Abbreviations: aa, amino acids; GFP, green fluorescent protein; ORF, open reading frame.

MATERIALS AND METHODS

Strains and Media—The *S. cerevisiae* strain GRF18 (*MATα his3-11,15 leu2-3,112*) served as a source of chromosomal DNA, strain EGY48 (*MATα his3 trp1 ura3 LexA*$_{op(x6)}$*-LEU2*) transformed by pSH18-34 was used for transactivation experiments, and strain No. 20376 (*MATa/α his3D1/his3D1 leu2D0/leu2D0 ura3D0/ura3D0 LYS2/lys2D0 MET15/met15D0 PRP45/prp45Δ::KanMX4*; ResGen™, Invitrogen, USA) was employed in complementation tests. The cells were cultured in YPD (1% yeast extract, 2% peptone, and 2% glucose) or SD [0.67% Yeast Nitrogen Base (Difco) and 2% glucose] media. The GFP-expressing cells were cultured in SD medium ± 1.0 mM methionine. Meiosis was induced during ~5 days of incubation on Fowell sporulation plates (0.5% KAc and 0.23% KCl), which was preceded by 24 h of growth on presporulation plates (0.5% yeast extract, 1% peptone, and 10% glucose). The haploids were checked for resistance to 200 μg/ml geneticin (G418; Amersham) on YPD plates. When required, nutritional supplements were added to the media. Yeast transformations were performed by the lithium acetate procedure (*21*).

Plasmid Constructions—The DNA fragment encoding full length Prp45 (379 aa) was amplified from chromosomal DNA using primers 5′-GCTGAATTCA TGTTTAGTAA CAGACTACC-3′ (1) and 5′-GCCGAATTCA ACTCAAGCAC AAGAATG-3′ (2). This and all subsequent PCR reactions were performed with Expand™ High Fidelity polymerase (Roche Diagnostics). The product was cloned into the *Eco*RI site of pBluescript SK(+) and sequenced. Truncated versions, *PRP45*(1–330), *PRP45*(1–261), *PRP45*(1–190), *PRP45*(53–190), *PRP45*(123–190), and *PRP45*(254–379), were obtained by PCR amplification of the original sequence using the primers (1) and 5′-GATGAATTCC TAGACGAACA GTGGGTTG-3′ (3), (1) and 5′-GAACTCGAGC TAAACTATTG CTCCCGTC-3′ (4), (1) and 5′-GAACTCGAGC TAAAGAGCTT TACCTACAC-3′ (5), 5′-GCTGAATTCA TGGATCCATT GTTGCC-3′ (6) and (5), 5′-GCTGAATTCA TGCTATCGGT TCCTTTGCC-3′ (7) and (5), and 5′-GCCGAATTCA TGGGAGCAAT AGTTAAGC-3′ (8) and 5′-GGACTCGAGC TAGGCGCCAT AGTTATC-3′ (9), respectively. The products were cut by either *Eco*RI or *Eco*RI and *Xho*I and cloned into *Eco*RI- or *Eco*RI- and *Xho*I-digested pEG202 (*22*), which allowed the expression of Prp45 and its deletion derivatives as N-terminally LexA-tagged fusion proteins. To obtain LexA-Prp45(1–119), the *Bam*HI fragment was deleted from pLexA-Prp45.

The mutation in the SNW motif SNW170-172AAA was generated in the full length Prp45 [Prp45(170AAA)] using the primer 5′-CCTGCAGCTG TGTCAGCGGC CGCAAATCCA AATGGTTATAC-3′ (10) and the Altered Sites® II *in vitro* Mutagenesis System according to the manufacturer's instructions (Promega, USA). The mutation was verified by DNA sequencing (AGOWA, Germany).

The construct encoding N-terminally His$_6$-tagged Prp45 (pHis-Prp45) was obtained PCR with the primers 5′-GATGGTACCA TGTTTAGTAA CAGACTACCAC-3′ (11) and 5′-GGTCTCGAGC AGCACAAGAA TGCTTTGTT-3′ (12). The product was cut by *Kpn*I and *Xho*I, ligated into *Kpn*I- and *Xho*I-cleaved plasmid pDXA-HC (*23*), recut by *Hin*dIII and *Xho*I, and inserted into *Hin*dIII- and *Xho*I- digested centromeric pADH416 (*24*). The plasmids pHis-Prp45(1–330), pHis-Prp45(1–261), pHis-Prp45(1–190), and pHis-Prp45(1–119) were generated from pHis-Prp45 by replacing its *Sac*I-*Xho*I fragment with the respective deletion derivatives. To express Prp45(53–190), Prp45(123–190), Prp45(254–379), and Prp45(170AAA), the corresponding *Eco*RI-*Xho*I fragments were transferred from pEG202 to pADH416.

To prepare yEGFP3-tagging expression plasmids, the fragments encoding the Prp45 and its deletion derivatives were cut by either *Eco*RI or *Eco*RI and *Xho*I from pEG202 and ligated in frame into *Eco*RI- or *Eco*RI- and *Xho*I-digested pUG36 vector (*25*).

Reporter Gene Assay—Transcriptional activation by LexA-Prp45 and its deletion derivatives was measured as β-galactosidase production in strain EGY48 using pSH18–34 as the reporter plasmid (*22*). Cells were shaken overnight in SD medium, diluted to OD_{600} = 0.1 and incubated until they reached OD_{600} = 1.0. Cells from 3 ml of culture were collected, cooled on ice, and washed once each with 5 ml of KP buffer (100 mM potassium phosphate; pH 7.8) and KP buffer containing 5% glycerol and protease inhibitor mix (2 μg/ml each of leupeptin, aprotinin, and pepstatin). The cell pellet was reconstituted with 40 μl of the latter buffer and glass beads were added (1.5 g; 425–600 μm, Sigma). The suspension was vortexed vigorously for 2 min at 4°C, incubated for 30 min with additional Triton X-100 (0.2% final concentration), and centrifuged at 13,000 ×*g* for 5 min. The β-galactosidase activity in the supernatant was estimated using the chemiluminiscent assay AURORA™ Gal-XE (ICN Pharmaceuticals) according to manufacturer's recommendations in a scintillation counter. Protein concentration was determined by the Bio-Rad Protein Assay (Bio-Rad) and Prp45 expression was monitored by immunodetection.

Immunodetection—Total cell extract was prepared from mid-log cells grown in SD medium. Cells corresponding to 6 ml of culture at OD_{600} ~0.5 were collected, washed with 1 ml of water and frozen at –20°C for 15 min. The pellet was vigorously vortexed with 100 μl of 1.85 M NaOH containing 7% β-mercaptoethanol, and after 10 min, 100 μl of 50% trichloroacetic acid was added. After an additional 5 min, the precipitate was pelleted (13,000 ×*g*, 10 min) and washed briefly with 500 μl of 1M Tris (*26*). All incubations and solutions were kept at 0°C. The proteins were transferred into Laemmli buffer, separated on 10% SDS-PAGE gels and transferred onto a nitrocellulose membrane (Bio-Rad). Immunochemical detection of His$_6$-tagged and LexA-tagged Prp45 was performed using monoclonal anti-His (HIS-1, Sigma) and anti–LexA IgG1 (2–12, Santa Cruz Biotechnology) antibodies, respectively, and anti–mouse IgG1 coupled with alkaline phosphatase (Bio-Rad).

Complementation Tests—To perform random spore analysis, the cells were brought to sporulation, treated with 3% *Helix pomatia* hepatopancreas juice, and then mildly sonicated with several short pulses. The spores were counted under a microscope and spread on selective SD plates. The haploids (lysine and/or methionine auxotrophs) were tested for G418 resistance. The absence of PRP45 in the chromosome of G418-resistants was confirmed by PCR. To perform tetrade analysis, the sporangia were pre-treated in hepatopancreas juice for *ca.* 30 min and dissected on YPD plates using a micromanipulator. The spores were allowed to germinate and grow for 2–3 days. Afterwards, the corresponding colonies were picked from the master plates, trans-

ferred into the wells of a 96-well plate and replica-spotted in duplicates onto YPD-plates with or without G418 using a custom-designed 48-pin spotter.

RESULTS

Complementation Analysis of Prp45—The essential Prp45 of *S. cerevisiae* is a 379–amino acid gene product lacking at the N-terminus, apart from all known orthologs, ca 80 amino acids containing a highly-conserved G-rich box. Also, the SH3/WW binding site in the central part of the primary sequence and some additional motifs are not conserved. On the other hand, the absolutely conserved "SNWKN" sequence, the helical segment and the SH2-like domain are present (Fig. 1) (*29*). In human SKIP, these regions were assigned a number of binding partners.

In preliminary experiments, we employed the LexA-tagging multicopy expression plasmid pEG202 to achieve the expression of Prp45 and its deletion derivatives in the *PRP45/prp45* diploid strain. Sporulants showing lysine and/or methionine auxotrophy were examined for resistance to G418. The ratios of resistant to sensitive haploids harboring pLexA-Prp45(1–330), pLexA-Prp45(1–261), and pLexA-Prp45(1–190) were ~1:1, which suggests that the C-terminal portion of Prp45, starting at position 191, is dispensable for survival. The presence and size of the recombinant proteins in the diploids was confirmed using anti-LexA specific antibodies (Fig. 2B).

We next expressed His_6-tagged Prp45 as well as the deletion-derivatives in the *PRP45/prp45* diploid employing a meiotically stable centromeric plasmid. The effects of both the C-terminal and N-terminal truncations on the ability of the recombinant protein to fulfile the essential function of wild-type Prp45 were tested employing a tetrade analysis. The haploid segregants were checked for their ability to sustain the antibiotic G418. As documented in Fig. 2A, the His_6-tagged Prp45 and His_6-Prp45(1–330), His_6-Prp45(1–261), His_6-Prp45(1–190), and Prp45(170AAA) supported growth of all four spores (upper panels), two of which were G418-resistant (lower panels). The cells expressing His_6-Prp45(1–119), as well as Prp45(53–190), Prp45(123–190), and Prp45(254–379), gave rise to two viable G418-sensitive segregants. We observed the manipulated spores during the first 72 h of cultivation. The non-complemented *prp45* spores gave rise to microcolonies of several small cells (~4–16), after which growth stopped (data not shown). The results summarized in Fig. 2C show that the N-terminal half of Prp45 alone is sufficient to fulfill the essential function of wild-type Prp45.

Prp45 is a predicted nuclear protein (*9*), which is in agreement with the nuclear localization of human SKIP (*16*), *Dictyostelium* SnwA and *S. pombe* Snw1p (unpublished). The failure of some of the truncated Prp45 variants to complement the knockout could be due to defects in their proper compartmentalization, *e.g.* because of the deletion of nuclear localization sequences. We constructed GFP-tag-

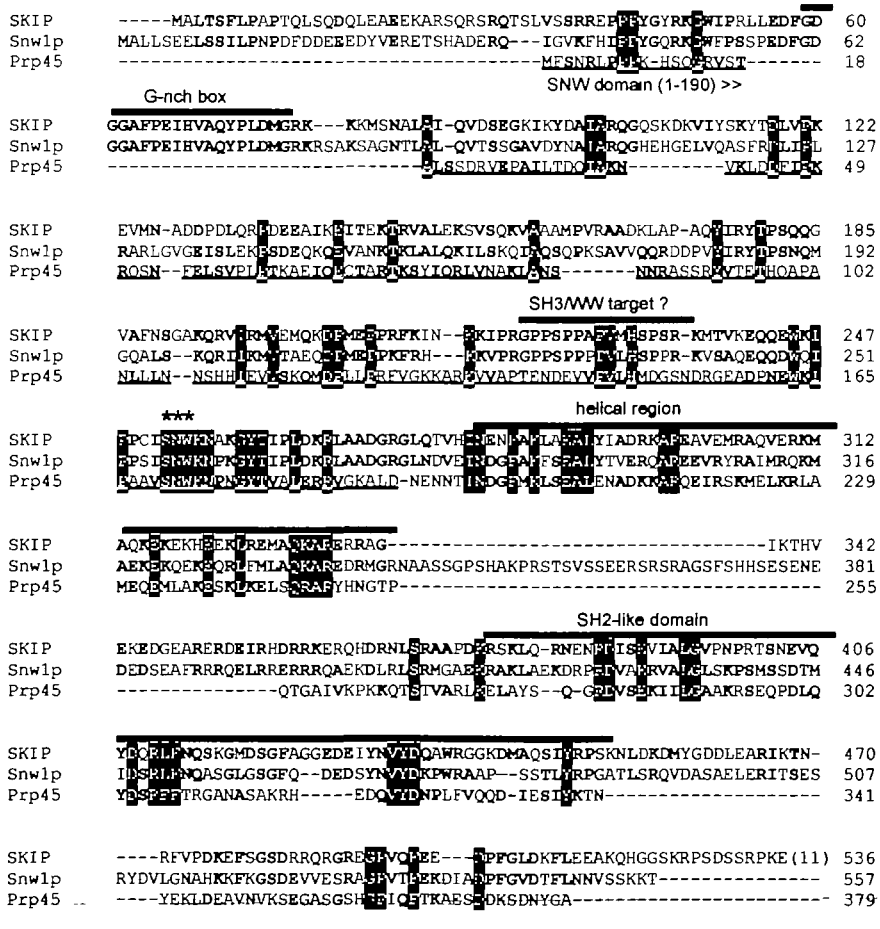

Fig. 1. **The alignment of *S. cerevisiae* Prp45 with the human and *S. pombe* orthologs, SKIP (NP_036377) and Snw1p (Q09882), respectively.** The alignment was generated using Clustal W 1.81; overall identities are emphasized by reverse print boxes, and partial identities or conserved amino acids are in bold. The region of Prp45 denoted as the "SNW domain" is underlined and the mutation SNW170-172AAA is marked by asterisks.

ging expression plasmids for all the Prp45 variants and employed GFP-tagged Prp45, Prp45(1–190), and Prp(1–119) in complementation analysis. We reproduced the results of Fig. 2 (data not shown), which suggests that the addition of the N-terminal moiety does not interfere with the function of the protein. The examination of GFP-Prp45 expressing cells *in vivo* revealed "speckled" nuclear localization over a diffuse nuclear signal, but almost no cytosolic fluorescence (Fig. 3). The comparison of low signal- to high signal-producing cells showed no difference in fluorescence distribution. We found that while the distribution of GFP-Prp45(1–330) between the nucleus and cytosol was indistinguishable from that of the full length GFP-Prp45, the distribution of GFP-Prp45(1–261) as well as other truncated variants was shifted towards the cytosol. Among them, GFP-Prp45(1–261) and GFP-Prp45(254–379) were the only variants exhibiting nuclear enrichment of the signal as compared to the distribution of free GFP (Fig. 3). Clearly however, truncated Prp45, including the non-complementing variants, was not excluded from the nuclear compartment. The behavior of GFP-Prp45(170AAA) was indistinguishable from its wild-type counterpart.

The growth of *prp45* cells harboring the complementing C-terminal truncations of Prp45 [LexA-Prp45(1–330), LexA-Prp45(1–261), and LexA-Prp45(1–190)] or LexA-Prp45 was measured at 20, 30, and 37°C. None of the conditions tested produced any significant variations in the growth rates of the transformants, indicating that the C-terminal 189 amino acids are fully dispensable for growth (data not shown).

DNA Targeted Prp45 Activates the Transcription of an Adjacent Marker Gene—Human SKIP was shown to coactivate VDR/RXR (*10*) or, among others, CBF1, and, at the same time, was proven to interact with corepressor complexes that inhibit signaling *via* these transcription factors (*14*). We found that the SKIP orthologs Snw1p and SnwA activated reporter genes in budding yeast when targeted to promoter sequences by a specific DNA-binding domain

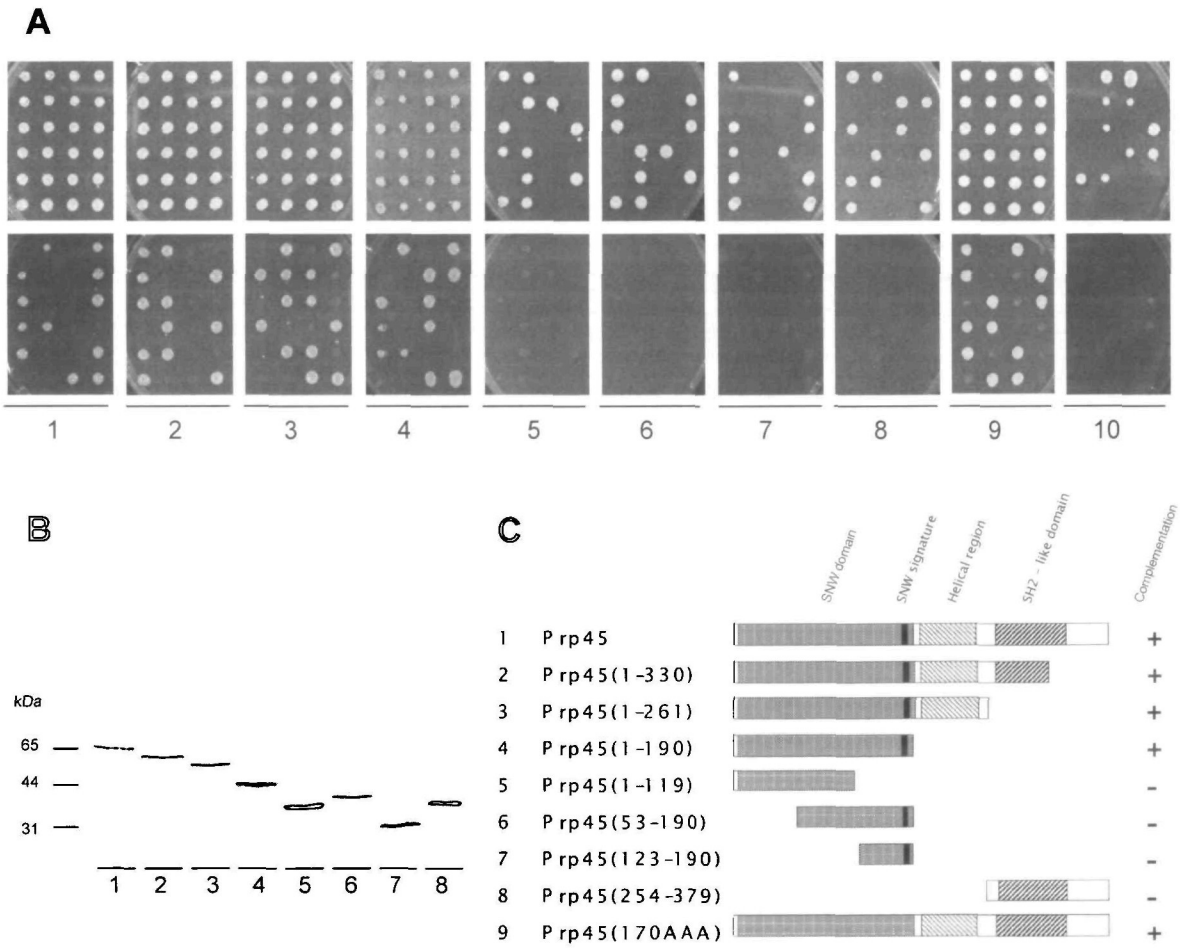

Fig. 2. **Complementation analysis of Prp45 and the examination of LexA-Prp45 transactivation potential.** (A) Tetrade analysis of the *PRP45/prp45* strain expressing various deletions of Prp45. Each pair of corresponding panels represents an example of the analysis of six independent tetrads of spores harboring the plasmid expressing His_6-Prp45 (1), His_6-Prp45(1–330) (2), His_6-Prp45(1–261) (3), His_6-Prp45(1–190) (4), His_6-Prp45(1–119) (5), Prp45(53–190) (6), Prp45(123–190) (7), Prp45(254–379) (8), Prp45(170AAA) (9), and the empty plasmid (pADH416) (10). The YPD plates without and with G418 are shown in the upper and lower panels, respectively. (B) Western analysis of the LexA-tagged Prp45 protein expressed in the *PRP45/prp45* strain. Total protein extracts were prepared and the expression of LexA-Prp45 (1), LexA-Prp45(1–330) (2), LexA-Prp45(1–261) (3), LexA-Prp45(1–190) (4), LexA-Prp45(1–119) (5), LexA-Prp45(53–190) (6), LexA-Prp45(123–190) (7), and LexA-Prp45(254–379) (8) was detected by anti-LexA antibody. (C) Schematic diagram summarizing the complementation experiments.

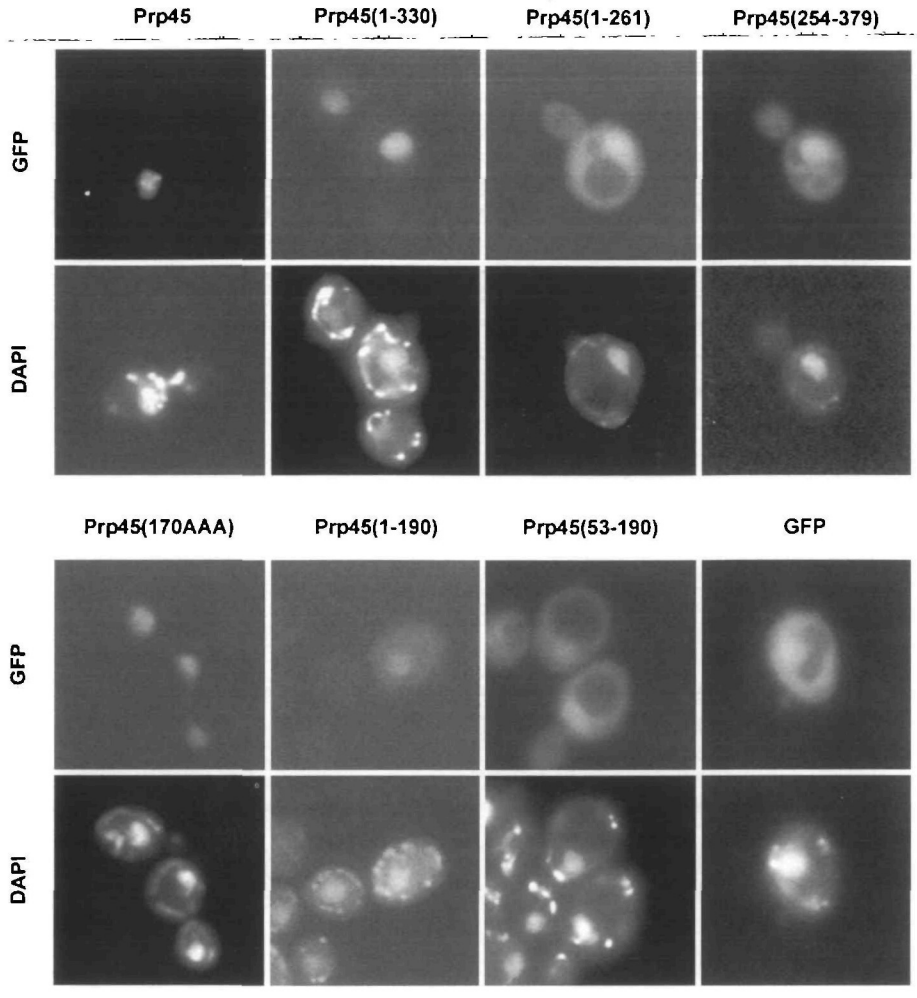

Fig. 3. **Subcellular localization of Prp45.** The EGY48 cells were transformed with plasmids expressing GFP-tagged variants of Prp45 or free GFP as indicated, propagated in shaken culture at low density in SD medium containing 1.0 mM methionine, and incubated in methionine-free medium before observation. After in vivo staining by DAPI, the cells were examined under a fluorescence microscope (Olympus BX60) using LUCIA 3.0 image analysis software (Laboratory Imaging, Prague).

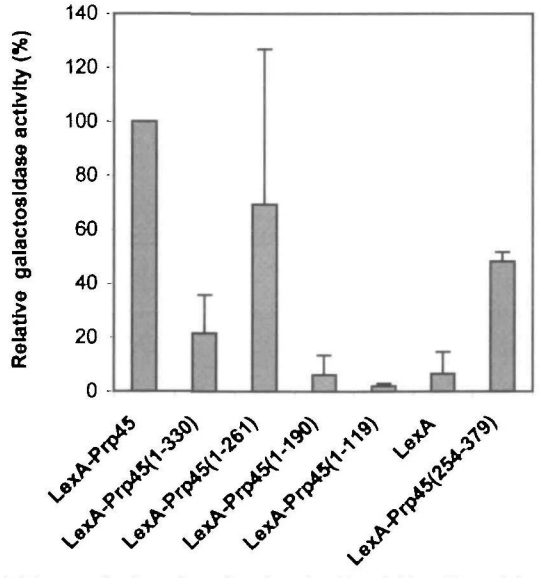

Fig. 4. **Transcriptional activation by LexA-Prp45 and its deletion derivatives.** The ability of LexA-tagged Prp45 and its truncated variants to activate transcription from a reporter plasmid pSH18-34 was measured as β-galactosidase activity in cell extracts (see "MATERIALS AND METHODS"). The enzymatic activity was normalized with respect to total protein content and expressed as relative β-galactosidase activity to compare independent experiments. Results are expressed as means ± SD (n = 4–6).

(unpublished), and a similar propensity was reported for SKIP in COS-7 cells (*27*). These findings led us to ask if the autologous Prp45 would also affect reporter gene activity when brought in contact with its promoter region.

We took advantage of a reporter system that uses β-galactosidase ORF positioned downstream of a minimal promoter with 8 LexA binding sites (*22*). The LexA-tagged Prp45 variants were expressed in the reporter strain and the accumulation of β-galactosidase was measured by chemiluminiscent assay in native cell extracts. LexA-Prp45 potentiated the reporter gene activity to ~30 times above the LexA-background, and this ability was retained, at least partly, by Prp45(1–261) as well as by the C-terminal domain [LexA-Prp45(254–379)]. The ability disappeared with the removal of the helical region [Prp45(1–190); Fig. 4]. In complementary experiments, we employed a yeast reporter system for transcription-repressing activity (*28*) and found that promoter-targeted Prp45 did not negatively influence ongoing transcription (data not shown). The results demonstrate that Prp45 is able to activate transcription when brought into the vicinity of the transcription start site.

DISCUSSION

We employed the *PRP45/prp45* strain and a set of *PRP45*-deletion derivatives to ask which part of Prp45 is critical

for functional complementation in the *prp45* haploid. Our results localized the essential function to the N-terminal half of the protein [Prp45(1–190); Fig. 2]. Prp45(1–190) suppressed *prp45* but it failed to potentiate transcription when targeted to a promoter [LexA-Prp45(1–190); Fig. 4]. GFP-tagged Prp45 and Prp45(1–330) were localized intranuclearly, whereas the other truncated versions, both complementing and non-complementing, showed only some nuclear enrichment (Fig. 3).

The N-terminal deletants Prp45(53–190) and Prp45-(123–190) lost the ability to complement, which might reflect the structural integrity of the N-terminal part as such or suggest folding problems. The N-terminus itself (amino acids 1 to 52) is relatively poorly conserved, but the region between amino acids 53 and 123 is 42% similar to human SKIP. A corresponding region in the *Dictyostelium* SnwA and the *Schizosaccharomyces* Snw1p binds the small cyclophilin of the CypE/Cyp2p family (PPIL1/CGI-124 in human) (*30*).

The C-terminal deletions were chosen (a) to correspond with the truncation of human SKIP that abolishes the ability of the protein to coactivate VDR [Prp45(1–330)] (*10*), (b) to eliminate the SH2-like domain implicated in the self association of Snw1p [Prp45(1–261)] (*18*), (c) to remove the helical motifs, which were proven to interact with VDR and RXR [Prp45(1–190)] (*31*), and (d) to delete the region with the highest degree of conservancy among the orthologs [Prp45(1–119)]. None but the (d) deletion hampered the ability of the corresponding construct to support viability in the *prp45* haploid (Fig. 2). We propose the use of the denotation "SNW domain" for the first 190 amino acids of Prp45, which would correspond to 274 amino acids of SKIP. The overlapping region of SKIP binds Smad2/3 factors (aa 201–333) (*11*) and interacts with Ski (aa 176–292) (*13*). Notably, the results of budding yeast large-scale two-hybrid screens contained no homologs of the interactors identified for SKIP, SnwA, or Snw1p (*7, 8*).

The ortholog of Prp45 from *S. pombe* is also essential (*18*), which suggests that SNW proteins serve an equivalent vital function in higher eukaryotes. SnwA of *D. discoideum* did not, however, complement the *PRP45* knockout (data not shown). This is remarkable, given that (a) the domains added to the N-terminus are compatible with the function of the protein, (b) only the amino-terminal 190 amino acids of Prp45 are indispensable, and (c) most of the amino-terminal part of Prp45 is highly conserved. While it is possible that the SnwA-specific N-terminus has a dominant negative effect, the experiments also support the hypothesis that the essential function of Prp45 in *S. cerevisiae* requires a species-specific structure encoded within amino acids 1 and 190. Our newest results are in agreement with the latter suggestion. The mutagenesis of the absolutely conserved SNWKN motif in full length Prp45 (SNW170-172AAA) neither hampers the ability of the protein to suppress *prp45* nor influences its nuclear targeting.

We found that the DNA-targeted LexA-Prp45 fusion protein activated the transcription of the *lacZ* reporter gene positioned downstream of eight LexA binding sites. This ability indicates that the occurrence of the protein would lead to the potentiation of transcription from targeted promoters *in vivo*. Deletion analysis mapped the activity to the conserved helical segment (aa 190–261) together with the basic C-terminal domain (aa 254–379). The conclusion that the targeting of SNW protein to a promoter activates transcription of the downstream gene is also in agreement with the observation that the DNA-targeted carboxyl-terminus of SKIP activates the reporter in COS-7 cells (*27*).

The C-terminal 190 amino acids of Prp45, corresponding to the VDR/RXR-binding and transcription-activating region in SKIP, and including the predicted helices, can be deleted without compromising the ability of Prp45 to fulfill its essential function. Does the C-terminal part act merely as a go-between, linking additional regulators, the dysfunction of which is not lethal, to an essential, perhaps cell cycle-related, task? According to this view, the recruitment of SKIP by a wide array of transcription factors may point to some basic nuclear function that these regulators must employ in order to modulate transcription. The identification of the essential part of Prp45 should help to trace the key cellular role of the more complex SKIP ortholog.

We thank Prof. B. Janderova and Prof. J. Forstova for the use of the micromanipulator facility and fluorescence imaging system, respectively.

REFERENCES

1. Hirose, Y. and Manley, J.L. (2000) RNA polymerase II and the integration of nuclear events. *Genes Dev.* **14**, 1415–1429
2. Ogryzko, V.V. (2001) Mammalian histone acetyltransferases and their complexes. *Cell. Mol. Life Sci.* **58**, 683–692
3. Lemon, B. and Tjian, R. (2000) Orchestrated response: a symphony of transcription factors for gene control. *Genes Dev.* **14**, 2551–2569
4. Ge, H., Si, Y., and Wolffe, A.P. (1998) A novel transcriptional coactivator, p52, functionally interacts with the essential splicing factor ASF/SF2. *Mol. Cell* **2**, 751–759
5. Diehl, B.E. and Pringle, J.R. (1991) Molecular analysis of *Saccharomyces cerevisiae* chromosome I: identification of additional transcribed regions and demonstration that some encode essential functions. *Genetics* **127**, 287–298
6. Harris, S.D., Cheng, J., Pugh, T.A., and Pringle, J.R. (1992). Molecular analysis of *Saccharomyces cerevisiae* chromosome I. On the number of genes and the identification of essential genes using temperature-sensitive-lethal mutations. *J. Mol. Biol.* **225**, 53–65
7. Ito, T., Chiba, T., Ozawa, R., Yoshida, M., Hattori, M., and Sakaki, Y. (2001) A comprehensive two-hybrid analysis to explore the yeast protein interactome. *Proc. Natl. Acad. Sci. USA* **98**, 4569–4574
8. Uetz, P., Giot, L., Cagney, G., Mansfield, T.A., Judson, R.S., Knight, J.R., Lockshon, D., Narayan, V., Srinivasan, M., Pochart, P., Qureshi-Emili, A., Li, Y., Godwin, B., Conover, D., Kalbfleisch, T., Vijayadamodar, G., Yang, M., Johnston, M., Fields, S., and Rothberg, J.M. (2000) A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. *Nature* **403**, 623–627
9. Albers, M. and Beggs, J. (1999) Identification and characterisation of Prp45p and Prp46p, two novel pre-mRNA splicing factors in *Saccharomyces cerevisiae*. http://genome-www.stanford.edu/Saccharomyces/ (12.12.2001)
10. Baudino, T.A., Kraichely, D.M., Jefcoat, S.C., Winchester, S.K., Partridge, N.C., and MacDonald, P.N. (1998) Isolation and characterization of a novel coactivator protein, NCoA-62, involved in vitamin D-mediated transcription. *J. Biol. Chem.* **273**, 16434–16441
11. Leong, G.M., Subramaniam, N., Figueroa, J., Flanagan, J.L., Hayman, M.J., Eisman, J.A., and Kouzmenko, A.P. (2001) Ski-interacting protein interacts with Smad proteins to augment transforming growth factor-beta-dependent transcription. *J. Biol. Chem.* **276**, 18243–18248
12. Dahl, R., Wani, B., and Hayman, M.J. (1998) The Ski oncopro-

tein interacts with Skip, the human homolog of *Drosophila* Bx42. *Oncogene* **16**, 1579–1586
13. Prathapam, T., Kuhne, C., Hayman, M., and Banks, L. (2001) Ski interacts with the evolutionarily conserved SNW domain of Skip. *Nucleic Acids Res.* **29**, 3469–3476
14. Zhou, S., Fujimuro, M., Hsieh, J.J., Chen, L., and Hayward, S.D. (2000) A role for SKIP in EBNA2 activation of CBF1-repressed promoters. *J. Virol.* **74**, 1939–1947
15. Zhou, S., Fujimuro, M., Hsieh, J.J., Chen, L., Miyamoto, A., Weinmaster, G., and Hayward, S.D. (2000) SKIP, a CBF1-associated protein, interacts with the ankyrin repeat domain of NotchIC To facilitate NotchIC function. *Mol. Cell. Biol.* **20**, 2400–2410
16. Neubauer, G., King, A., Rappsilber, J., Calvio, C., Watson, M., Ajuh, P., Sleeman, J., Lamond, A., and Mann, M. (1998) Mass spectrometry and EST-database searching allows characterization of the multi-protein spliceosome complex. *Nat. Genet.* **20**, 46–50
17. Mintz, P.J., Patterson, S.D., Neuwald, A.F., Spahr, C.S., and Spector, D.L. (1999) Purification and biochemical characterization of interchromatin granule clusters. *EMBO J.* **18**, 4308–4320
18. Ambrozkova, M., Puta, F., Fukova, I., Skruzny, M., Brabek, J., and Folk, P. (2001) The fission yeast ortholog of the coregulator SKIP interacts with the small subunit of U2AF. *Biochem. Biophys. Res. Commun.* **284**, 1148–1154
19. Candau, R., Zhou, J.X., Allis, C.D., and Berger, S.L. (1997) Histone acetyltransferase activity and interaction with ADA2 are critical for GCN5 function in vivo. *EMBO J.* **16**, 555–565
20. Poux, S., McCabe, D., and Pirrotta, V. (2001) Recruitment of components of Polycomb Group chromatin complexes in *Drosophila*. *Development* **128**, 75–85
21. Gietz, D., St. Jean, A., Woods, R.A., and Schiestl, R.H. (1992) Improved method for high efficiency transformation of intact yeast cells. *Nucleic Acids Res.* **20**, 1425
22. Golemis, E.A., Gyuris, J., and Brent, R. (1996) Interaction hunt/two-hybrid systems to identify interacting proteins in *Current Protocols in Molecular Biology* (Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.D., Smith, J.A., and Struhl, K., eds.) John Willey & Sons, New York
23. Manstein, D.J., Schuster, H.P., Morandini, P., and Hunt, D.M. (1995) Cloning vectors for the production of proteins in *Dictyostelium discoideum*. *Gene* **162**, 129–134
24. Mumberg, D., Muller, R., and Funk, M. (1995) Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. *Gene* **156**, 119–122
25. Niedenthal, R.K., Riles, L., Johnston, M., and Hegemann, J.H. (1996) Green fluorescent protein as a marker for gene expression and subcellular localization in budding yeast. *Yeast* **12**, 773–786
26. Horvath, A. and Reizman, H. (1994) Rapid protein extraction from *Saccharomyces cerevisiae*. *Yeast* **10**, 1305–1310
27. MacDonald, P.N., Baudino, T.A., Tokumaru, H., Dowd, D.R., and Zhang, C. (2001) Vitamin D receptor and nuclear receptor coactivators: crucial interactions in vitamin D-mediated transcription. *Steroids* **66**, 171–176
28. Tzamarias, D. and Struhl, K. (1994) Functional dissection of the yeast Cyc8-Tup1 transcriptional co-repressor complex. *Nature* **369**, 758–761
29. Folk, P., Puta, F., Krpejsova, L., Blahuskova, A., Markos, A., Rabino, M., and Dottin, R.P. (1996) The homolog of chromatin binding protein Bx42 identified in *Dictyostelium*. *Gene* **181**, 229-231
30. Skruzny, M., Ambrozkova, M., Fukova, I., Martinkova, K., Blahuskova, A., Hamplova, L., Puta, F., and Folk, P. (2001) Cyclophilins of a novel subfamily interact with SNW/SKIP coregulator in *Dictyostelium discoideum* and *Schizosaccharomyces pombe*. *Biochim. Biophys. Acta* **1521**, 146–151
31. Zhang, C., Baudino, T.A., Dowd, D.R., Tokumaru, H., Wang, W., and MacDonald, P.N. (2001) Ternary complexes and cooperative interplay between NCoA-62/Ski-interacting protein and steroid receptor coactivators in vitamin D receptor-mediated transcription. *J. Biol. Chem.* **276**, 40614–40620