Isolation of a novel complex of the SWI/SNF family from *Schizosaccharomyces pombe* and its effects on in vitro transcription in nucleosome arrays

Giuliano Bernal · Edio Maldonado

Abstract The family of ATP-dependent chromatin-remodeling factors plays a central role in eukaryotic transcriptional regulation. These complexes can alter the structure of chromatin by mechanisms that involve nucleosome sliding, dissociation, or replacement over a specific promoter. The SWI/SNF chromatin-remodeling complex is required for transcriptional activation or repression in a subset of genes. In the present study we have isolated the spSWI/SNF complex from *Schizosaccharomyces pombe*, which has at least seven subunits among them spSwi1-like and the catalytic subunit spBrg1. These subunits are homologues to Swi1 and Swi2/Snf2, respectively in *Sacharomyces cerevisiae*. Moreover, we have demonstrated that spSWI/SNF is able to promote in vitro transcription by RNA polymerase II (RNAPII) in a reconstituted system. In our transcription assays with cellular extracts of *Sc. pombe* we did not observe inhibition when α-Swi1 antibodies were utilized, indicating that other chromatin-remodeling complexes may allow transcription in *Sc. pombe.*

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Introduction

The eukaryotic genome is packed into nucleosomes, which are comprised of 146 bp of DNA wrapped around a core protein composed of two copies of the four histones H3, H4, H2A, and H2B [1, 2]. A fifth histone H1 and other non-histone proteins, induce a compressed state of the chromatin [3], which makes it inaccessible to the transcriptional machinery, silencing the genes within it [4–6] In eukaryotic cells, the transcriptional machinery is formed of RNA polymerase II (RNAPII), the general transcription factors (GTFs) TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH, and a variety of protein activators, co-activators and chromatin remodeling-complexes [7, 8].

The nucleosomes are able to repress the transcription in at least three different ways. First of all, they occlude DNA binding sites for protein, thereby interfering with the interaction of activator and repressor proteins, polymerases, and transcription factors [9]. Second, the nucleosome chain can coil and repress the transcription of a whole chromosomal domain [10]. Finally, the interactions of nucleosomes with additional chromosomal proteins in heterochromatin repress gene expression in a hereditary manner [11].

Therefore, complexes that modify the nucleosome's position on the promoter must be recruited, to prevent the repressive effect exerted by the nucleosome so that transcription can proceed. The chromatin remodeling is directed by two families of complexes, those that covalently modify nucleosomal histone proteins through acetylation,

phosphorylation, or methylation, and those that alter chromatin structure through hydrolysis of the energy-rich molecule adenosine triphosphate (ATP) [12]. This last family of remodelers can be divided into four classes according to different protein composition and function, and they include SWI/SNF, ISWI, Mi-2, and Ino80 [13, 14]. Moreover the SWI/SNF group can be subdivided into two, highly conserved subclasses. The first comprise yeast SWI/SNF (ySWI/SNF), BAP and mammalian BAF complexes; whereas the second subfamily includes yeast RSC, fly PBAP, and mammalian PBAF complexes [14].

The SWI/SNF complexes are formed by nine or more subunits, including conserved proteins [13]. These complexes can mobilize histones along DNA, which could directly break the histone–DNA interaction [15–17].

In this work we used the yeast *Sc pombe*, which differs from *S. cerevisiae* in some fundamental aspects of transcription, such as chromosome size, centromere structure, transcription, splicing, and cellular cycle control. This makes *Sc. pombe* more similar to metazoan than *S. cerevisiae* [18].

We propose that as in *S. cerevisiae, Drosophila* and humans, homologues to SWI/SNF complexes should exist in *Sc. pombe*, and these complexes could alter the chromatin structure, facilitating transcription by RNAPII.

Materials and methods

Histone purification

Histones were purified following a published protocol [19]. A cellular pellet was obtained from chicken blood and resuspended in buffer I (0.15 M NaCl, 15 mM sodium citrate, 10 mM buffer phosphate pH 7.2, 150 U/ml heparin) and centrifuged to 3,000 rpm. The resulting pellet was resuspended in buffer II (0.1 M KCl, 50 mM Tris–HCl pH 7.5, 1 mM $MgCl_2$, 0.5% Tritón X-100) and centrifuged. The pellet was then resuspended in buffer III (0.1 M KCl, 50 mM Tris–HCl pH 7.5,1 mM $CaCl_2$) and lysed with sonication at 20 W RMS. The supernatant was dialyzed overnight at 4°C in buffer IV (0.1 M buffer phosphate pH 6.7, 0.63 M NaCl) and centrifuged to 30,000 rpm. The supernatant was chromatographied on hydroxyapatite and histones were eluted with buffer IV plus 0.93, 1.2, and 2 M NaCl.

Nucleosome assembly on pG5-MLT vector

The plasmid pG5-MLT contains a G-less cassette of 390 bp. and 5 Gal4 sites, which are upstream of the TATA box. Each assembly contains 2 μg of pG5-MLT, 1.8 μg of native histones, 150 μg BSA, 3 mM ATP, 30 mM phosphocreatine, 0.2 μg creatine kinase, 5 mM $MgCl_2$, 50 mM KCl, 10 mM Hepes pH 7.5, 0.2 mM EDTA, 25% glycerol, and 0.3 μg of RSF (Remodeling and Spacing Factor, isolated from HeLa cells). The reaction was incubated for 6 h at 30°C in a volume of 150 μl. The assembled nucleosomes were then purified on a column of Sepharose CL-4B equilibrated with Buffer CL-4B (10 mM Hepes pH 7.8, 0.5 mM EDTA). The nucleosomes were eluted with the same buffer in aliquots of 500 μl.

Digestion with micrococcal nuclease (MNase)

About 2 μg of nucleosomes were digested with 1 U/μg DNA of MNase in 80 μl of nuclease buffer (10 mM Hepes pH 7.8, 5 mM $MgCl_2$, 50 mM KCl, 0.5 mM EDTA, 10% glycerol) and 3 mM $CaCl_2$ [20]. The micrococcal nuclease digestion reaction was carried out for 2 or 10 min at 30°C and stopped by the addition of 80 μl of stop buffer (20 mM EDTA, 0.2 M NaCl, 1% SDS). The DNA was then extracted with phenol–chloroform and resolved on a 6% polyacrylamide electrophoresis gel.

Preparation of *Sc. pombe* whole cell extract

Whole cell extracts were prepared from 500 g (wet weight) of wild-type strain 972 h that were obtained from 200 l of culture. The cells were mixed with liquid nitrogen in a 4 l Waring blender and blended four times for 5 min each at maximum speed. The broken cells were mixed with buffer A (100 mM Hepes pH 7.9, 250 mM KCl, 5 mM EGTA, 10 mM EDTA, 2.5 mM dithiothreitol [DTT], 1 mM phenylmethanesulfonyl fluoride [PMSF], 0.5 μg/ml Pepstatin A), and the mixture was centrifuged in a Sorvall rotor at 30,000*g*, at 4°C for 45 min. The supernatant was recovered and dialyzed against buffer B (20 mM Hepes pH 7.8, 2 mM DTT, 5 mM EGTA, 2 mM EDTA, 10 mM Mg_2SO_4, 1 mM PMSF, 20% glycerol). The extract was stored at –80°C until use.

Expression and purification of *Sc. pombe* TFIIE, TFIIF, TFIIB, and TBP

The transcription factors TBP, TFIIB, TFIIEα, TFIIEβ, TFIIFα, and TFIIFβ were expressed in *Escherichia coli* strain BL21 (DE3) and purified following Ref. [21].

Purification of *Sc. pombe* RNAPII holoenzyme and TFIIH

RNAPII holoenzyme was purified from a TAP-spMed7 *Sc. pombe* strain by following the TAP-tag purification

protocol. The cellular extract was made according to the procedure described above, and dialyzed against dialysis buffer (10 mM Tris–Cl pH 8.0, 150 mM NaCl, 0.1% NP-40). Then, 3 ml of the extract were mixed with 1 ml of IgG-Sepharose and incubated for 2 h at 4°C. The resin was washed with 5 volumes of dialysis buffer and then with 5 volumes of TEV buffer (50 mM Tris–HCl pH 8.0, 0.5 mM EDTA, 1% glycerol, 1 mM DTT). RNAPII holoenzyme was eluted incubating the resin with 500 U protease TEV for 1 h at 30°C. TFIIH was purified from a TAP-spTfb2 *Sc. pombe* in the same way as RNAPII holoenzyme.

Purification of RSF (remodeling and spacing factor)

RSF was purified from HeLa cell nuclear pellets following Ref. [2].

In vitro transcription assays

The conditions for the transcription reactions were based on those reported in Ref. [22]. The reaction mixtures contained proteins and vector pG5-MLT (relaxed or as nucleosomal arrays) as indicated in the legend of each figure. The transcription reactions were performed in 50 mM Hepes pH 7.8, 50 mM potassium glutamate, 15 mM magnesium acetate, 2.5 mM DTT, 1 U ribonuclease inhibitor (Promega), 10% glycerol, 0.4 mM ATP, 0.4 mM CTP, 0.4 μCi [α-^{32}P] UTP, 4 mM phosphoenolpiruvate, in a final volume of 30 μl for 30 min at 25°C. The transcription was stopped by the addition of 120 μl of 10 mM Tris–HCl, pH 7.5, 300 mM NaCl, 5 mM EDTA, 10 units RNase T1, and incubated at room temperature for 15 min. The reaction was treated with 12 μl of 10% SDS and 100 μg of proteinase K for 20 min at 30°C. The transcripts were ethanol-precipitated and analyzed by electrophoresis in a 6% polyacrylamide-7 M urea gel in TBE buffer. The gels were dried and analyzed by autoradiography.

Expression and purification of the C-terminal region of the spSwi1-like polypeptide

The sequence of this polypeptide was obtained from its homologue swi1 in *S. cerevisiae* (GenBank accession no. P09547) using the BLASTP program. The following primers were designed, FP: 5′-cccatatgcctctactcattcg-3′ and RP: 5′-ccggatccttaaatgccagat-3′, which amplify 870 bp corresponding to the C-terminal region of spSwi1-like. The forward primer contains a *Bam*H1 site and the reverse primer contains an *Nde*I site. The PCR product was cloned on TOPO-TA® (Invitrogen) and subcloned in-frame into the *Nde*I and *Bam*H1 sites of pET-15b. BL21(DE3) cells were then transformed and the protein expression was induced with IPTG. The recombinant protein was purified under denaturing conditions on a column of Ni-NTA agarose, as described by the manufacturer (Qiagen).

Preparation of antibodies against the polypeptide spSwi1-like

Rabbits were injected with 500 μg of the polypeptide in complete Freund's adjuvant, according to NIH protocols. Fifteen days later, another injection was applied in incomplete Freund's adjuvant. Serum from the rabbits was obtained 3 weeks later. The antibodies were immunopurified from the serum and used for Western Blot.

Purification of antibodies using spSwi1-like recombinant immobilized on a column of Ni-NTA agarose

The recombinant C-terminal region of the spSwi1-like was immobilized on a column of Ni-NTA agarose as described by the manufacturer (Qiagen), washed with Buffer C (8 M urea, 0.1 M NaH_2PO_4, 100 mM Tris–HCl pH 5.9) and equilibrated with TBS 1× (20 mM Tris–Cl pH 7.8, 0.3 M NaCl). The resin was incubated with serum for 1 h at 4°C, and then washed with TBS 1×, 0.1% NP-40. Antibodies were eluted with 4 M $MgCl_2$.

Purification of the complex spSWI/SNF from the cellular extract of *Sc. Pombe*

The complex spSWI/SNF was purified by the following steps of chromatography. First, the cellular extract of *Sc. pombe* was chromatographied on a DE-52 resin equilibrated with buffer G (20 mM Tris–HCl pH 7.8, 10% glycerol, 2 mM DTT, 0.1 mM PMSF), and supplemented with 0.05 M potassium acetate. The elution was made with 0.1 and 0.4 M potassium acetate in the same buffer. The fraction containing spSWI/SNF was then chromatographied on a column of phosphocellulose P11 and eluted with a gradient of 0.1–1.0 M potassium acetate in buffer G. The fraction containing spSWI/SNF was dialyzed with buffer phosphate (10 mM sodium phosphate pH 7.4, 2 mM DTT, 10% glycerol, 0.1 mM PMSF) and chromatographied on an hydroxyapatite column. The elution was done with a gradient of 10–400 mM buffer phosphate. The fraction containing the complex was finally chromatographied on a column of Q-Sepharose equilibrated with 0.1 M potassium acetate and eluted with a gradient of 0.1–0.6 M potassium acetate in buffer G. The purification was monitored using Western Blot against the spSwi1-like subunit.

Immunoprecipitation of the spSWI/SNF complex

The antibodies against the spSWI/SNF subunit, spSwi1-like, were immobilized on a protein A-agarose resin. The resin was then washed with sodium borate 0.2 M (pH 9.0) and incubated with 20 mM dimethyl pimelimidate for 30 min at room temperature and the reaction was stopped with Tris–HCl 1 M pH 7.8. The antibodies bound non-covalently were eluted with 100 mM Glycine–HCl pH 2.5. One hundred microliters of this resin were mixed with 100 µl of *Sc. pombe* cellular extract and incubated overnight at 4°C. The mix was centrifuged and the resin washed with buffer solution (Hepes 20 mM pH 8.0, 0.1 M KAc, EDTA 5 mM, glycerol 20%, 0.1 mM PMSF, 0.1% NP-40). The elution was made with urea 6 M, 10 mM Tris–HCl (pH 7.8) and the presence of spSWI/SNF was verified using SDS-PAGE 10% and Western blot against spSwi1-like and Brg1 (ATPase) subunits.

Results

Nucleosome assembly on pG5-MLT vector

Several different approaches have been used to assemble nucleosomes onto promoter-containing DNA for in vitro transcription studies. We assembled nucleosomes onto pG5-MLT plasmid using RSF, which can assemble regularly spaced nucleosome arrays in the presence of ATP. The assembled nucleosomes were purified on Sepharose CL-4B and used in the transcription assays. The partial micrococcal nuclease digestion showed the generation of mono, di, tri, and tetranucleosomes (Fig. 1). A 6% polyacrilamide gel is present where there is observed digestion of assembled plasmad pG5-MLT at different times (0, 2, and 10 min). At least four nucleosomes are shown in Lane 2, whereas only two visible nucleosomes in Lane 3 indicate an overdigestion of the plasmid. The plasmid pG5-MLT is shown in line 1 without digestion.

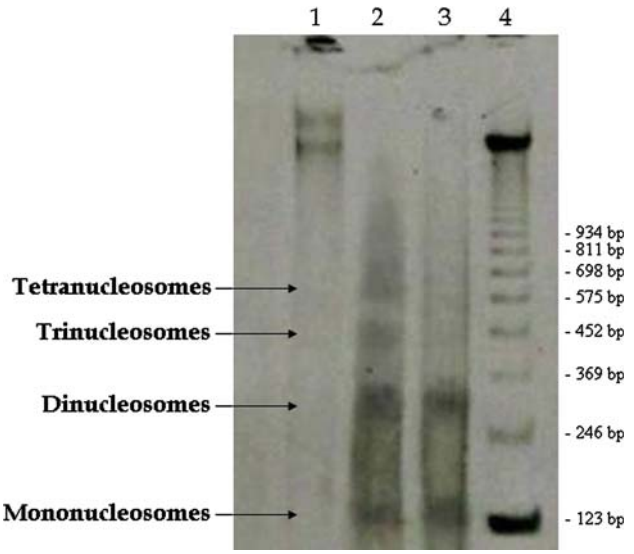

Fig. 1 A 6% SDS-PAGE gel shows the digestion of 0.5 µg of the pG5-MLT plasmid assembled in nucleosomal arrays and digested with 1.0 U MNase/µg DNA. In Lane 1, the plasmid without digestion is shown. The plasmid digested for 2 min with MNase is shown in Lane 2, and the overdigestion of the plasmid for 10 min with MNase is observed in Lane 3. Lane 4 corresponds to the DNA molecular weight marker

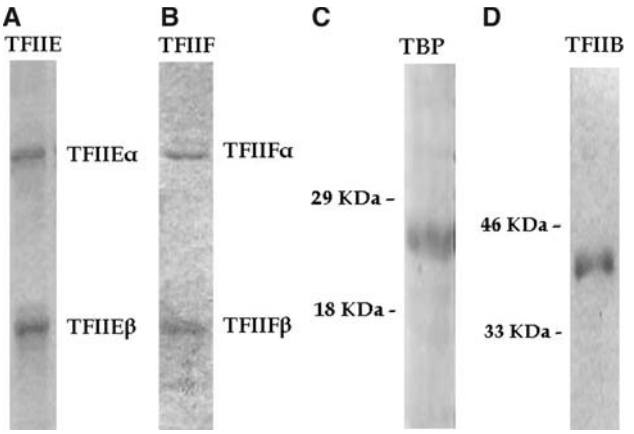

Fig. 2 This figure displays SDS-PAGE to 15% and stained with blue Coomasie and shows the purification of (**A**) TFIIE with its subunits TFIIEα (448 amino acids) and TFIIEβ (285 amino acids); (**B**) TFIIFα (490 amino acids) and TFIIFβ (307 amino acids); (**C**) TBP (26 kDa) and (**D**) TFIIB (38 kDa)

Expression and purification of *Sc. pombe* TFIIE, TFIIF, TFIIB, and TBP

Using the BLASTP tool, we identified *human and S. cerevisae* homologs of TFIIE, TFIIF, TFIIB, and TBP in *Sc. pombe*. The factors were cloned, expressed and purified, and were judged at least 90% pure by SDS/PAGE. *Sc. pombe* TFIIE is composed of two subunits, α (GenBank accession no. CAC32853) and β (GenBank accession no. CAC204446) (Fig. 2A). The *Sc. pombe* α subunit contains 434 amino acids and shares 21% amino acid sequence identity with its human homolog. *Sc. pombe* TFIIEβ contains 285 amino acids and shares 30% amino acid sequence identity with human TFIIEβ. TFIIF is also composed of two subunits, α (GenBank accession no. CAA22493) and β (GenBank accession no. NP595082) (Fig. 2B). *Sc. pombe* TFIIFα contains 490 amino acids but no homology with human TFIIFα was detected. *Sc. pombe* TFIIFβ has 301 amino acids and displays 27% identity with its human homolog. *Sc. pombe* TBP (GenBank accession no. P17871) contains 231 amino acids and shares 80% amino acid sequence identity with its human homolog and shows a

molecular weight of 26 kDa (Fig. 2C). *Sc. pombe* TFIIB (GenBank accession no. O13749) contains 340 amino acids and displays 44% identity with human TFIIB and a molecular weight of 38 kDa (Fig. 2D).

Purification of RNAPII holoenzyme and TFIIH

The RNAPII holoenzyme was purified by subjecting cell extracts made with the TAP-SpMed7 *Sc. pombe* strain to affinity chromatography on IgG beads. The TFIIH complex was purified in the same way from cells that express a TAP-p52. The identification of *Sc. pombe* TFIIH and RNAPII holoenzyme was done by SDS-PAGE 10% and stained with Coomasie Blue (Fig. 3A, B), and Western Blot against Srb4 (RNAPII holoenzyme) and p52 (TFIIH) (Data not shown).

Cloning and expression of the C-terminal region of the sp-Swi1-like polypeptide

Using the BLASTP tool in *S. cerevisiae* a homolog subunit of Swi1 was found for *Sc. pombe*. This protein contains 865 amino acids (GeneBank accession no. CAA20317) and shares 36% amino acid sequence identity with its *S. cerevisiae* homolog. Moreover, it displays a 27% amino acid sequence identity with human BAF250. We expressed a C-terminal region of this protein which was purified and electrophoresed on 15% SDS-PAGE and identified by Western Blot against the His-Tag (Fig. 4A, B, respectively). The C-terminal region of spSwi1-like possesses 35 kDa, meanwhile the wild spSwi1-like protein possesses a molecular weight of 96 kDa. This polypeptide was only used for antibody production.

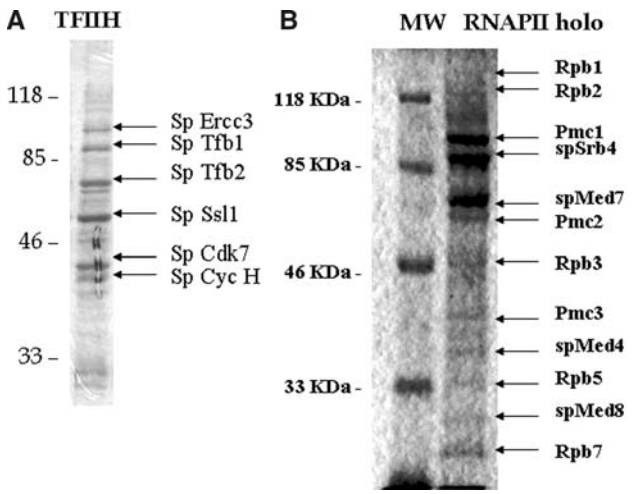

Fig. 3 This figure shows two SDS-PAGE gels with TFIIH (**A**) and RNAPII holoenzyme (**B**), respectively. Some subunits are shown in both figures

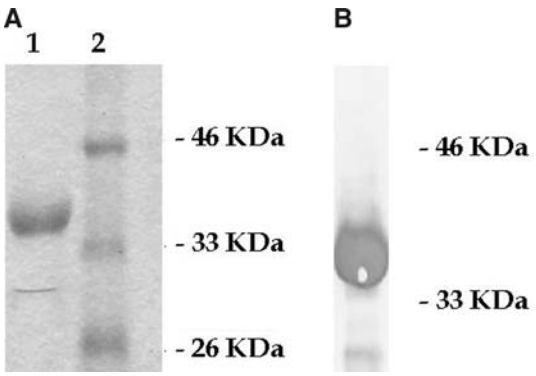

Fig. 4 Purification and identification of the C-terminal region of the spSwi1-like subunit of *Sc. pombe*. (**A**) 15% SDS-PAGE of the purified recombinant C-terminal region of the spSwi1-like polypeptide (1). In the Lane 2 the protein molecular weight is displayed. (**B**) Western Blot against the His-tag of the polypeptide observed in (**A**)

Purification and identification of the spSWI/SNF complex from the *Sc. pombe* extract

The spSWI/SNF complex was purified from an extract of *Sc. pombe* using several steps of standard chromatography (Fig. 5). The most purified fraction of the spSWI/SNF complex was obtained on the Q-Sepharose column eluted

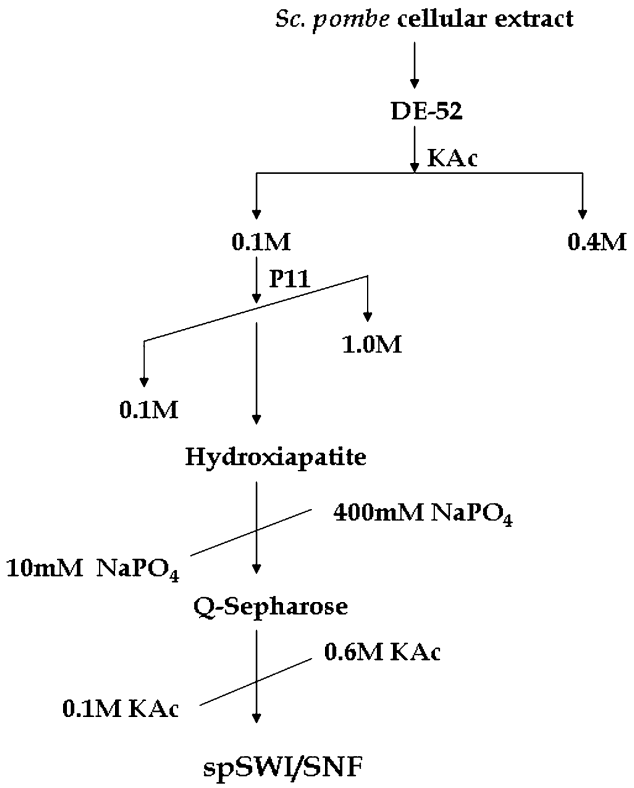

Fig. 5 Purification scheme of the spSWI/SNF complex from a cellular extract of *Sc. pombe*. Several steps of conventional chromatography were used and the purification was monitored using Western Blot against the spSwi1-like subunit

with approximately 0.2 M potassium acetate, and was subsequently used for in vitro transcription assays.

On the other hand, the spSWI/SNF complex was immunoprecipitated from an extract of *Sc. pombe* and showed at least seven subunits, two of them were identified using Western Blot. One subunit corresponds to spSwi1-like, and the other is spBrg1 (the ATPase subunit) which was identified using a human antibody, and possesses a molecular size of 148 kDa (Fig. 6B) and shares 62% amino acid sequence identity with human Brg1, from the BAF complex, and a 56% homology with yeast Snf2 belonging to the SWI/SNF complex in *S. cerevisiae*. This protein is significantly smaller than it's homologue in human and *S. cerevisiae*, however, the genome of *Sc. pombe* contains a gene that encodes for a conserved Brg-1-like protein (GeneBank accession no. BAD11105). The complex immunoprecipitated is shown in Fig. 6C and the seven subunits are indicated.

In vitro transcription assays

Based in previous unpublished assays, we investigated the effect of different *Sc. pombe* extract concentrations on the transcriptional activity on nucleosomal arrays. We found that the optimal concentration was between 2 µg and 5 µg of *Sc. pombe* extract in the assay (Fig. 7A, Lanes 1 and 2). High concentrations of the extract seriously diminished the transcriptional activity (Fig. 7A, Lanes 3–6). The control was a transcriptional assay with non-assembled plasmid pG5-MLT, where 50 µg of cellular extract of *Sc. pombe* efficiently generated a transcript (Fig. 7 A, Lane 7).

In order to investigate if the addition of an antibody to Swi1 could affect the transcription on naked DNA or nucleosomes, different concentrations of anti-Swi1 were added to the *Sc. pombe* extract in a transcriptional assay. Nevertheless the transcription was absolutely normal and no inhibition was observed (Fig. 7B, Lanes 7–11). The control was naked DNA and transcription inhibition was also absent (Fig. 7B, Lanes 1–5). Pre-immune serum was used as a control (Fig. 7B, Lanes 6 and 12). It is possible that other complexes in the *Sc. pombe* extract will allow transcription in nucleosome arrays in the absence of the spSWI/SNF.

Finally, we studied the effect of the spSWI/SNF complex in a reconstituted transcription assay. We incubated two different concentrations of spSWI/SNF with the RNAPII holoenzyme and the general transcription factors TBP, TFIIB, TFIIF, TFIIE, and TFIIH (Fig. 8). We found that 3 µg of spSWI/SNF stimulated transcription in nucleosomal arrays of DNA (Fig. 8, Lane 5) nevertheless, 6 µg inhibited the transcription in this assay (Fig. 8, Lane 6). Moreover, it is possible to observe that the basal transcriptional machinery was not sufficient for transcription in nucleosomal arrays (Fig. 8, Lane 4). When naked DNA was used, a robust transcript was observed (Fig. 8, Lane 1) and the addition of the fraction containing the spSWI/SNF complex did not have any effect on the transcriptional activity (Fig. 8, Lanes 2 and 3).

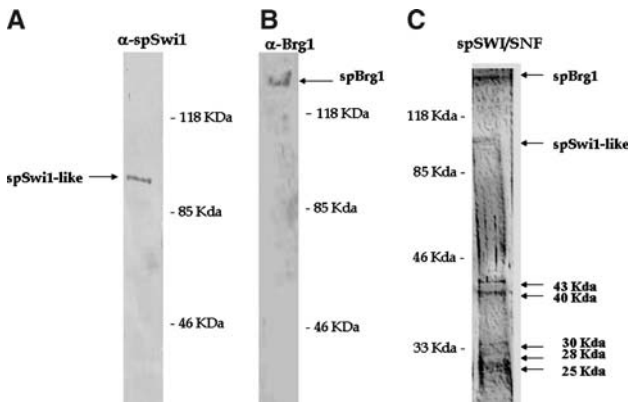

Fig. 6 Western Blot against the *Sc. pombe* subunits spSwi1-like (**A**) and spBrg1 (**B**), both of them presently in the immunoprecipitated spSWI/SNF complex. In (**C**) an SDS-PAGE to 10% with silver stains is shown where the immunoprecipitated spSWI/SNF complex is analyzed. In Lane 1 the complex of *Sc. Pombe* is shown. To the right of the figure two subunits are identified and others show their molecular weights

Discussion

In this work we have demonstrated the presence of a homolog of SWI/SNF complex in a cellular extract of *Sc. pombe*, which in suitable concentrations, allowed in vitro transcription in nucleosomal arrays.

Chromatin remodeling complexes have been purified from several organisms such as *S. cerevisiae, Drosophila*, and in humans [13, 14]. Its principal characteristic is possession of an ATP-dependent catalytic subunit, which can alter the nucleosome position [15, 17, 23, 24]. In this work we identify a subunit homolog to Brg1 (the catalytic subunit of human SWI/SNF) in a cellular extract fraction of *Sc. pombe* containing spSWI/SNF. For the identification we used human antibodies against Brg1, because the catalytic subunit of the SWI/SNF complex is very conserved, showing more than 50% amino acid sequence identity among them [13, 14]. Particularly, spBrg1 shared 62% homology with human Brg1 and 54% homology with *S. cerevisiae* Swi/Snf2. The catalytic subunit identified in the fraction, which contains spSWI/SNF, shows a molecular weight of 148 kDa and is smaller than their homologues in *S. cerevisiae, Drosophila*, or in humans. From an evolutional or functional point of view there is no clear answer for this result. Nevertheless, in other nucleosome remodeling complexes, like Iswi *(Drosophila)* or Ino80 *(S. cerevisiae)* the catalytic subunit has a smaller size [25].

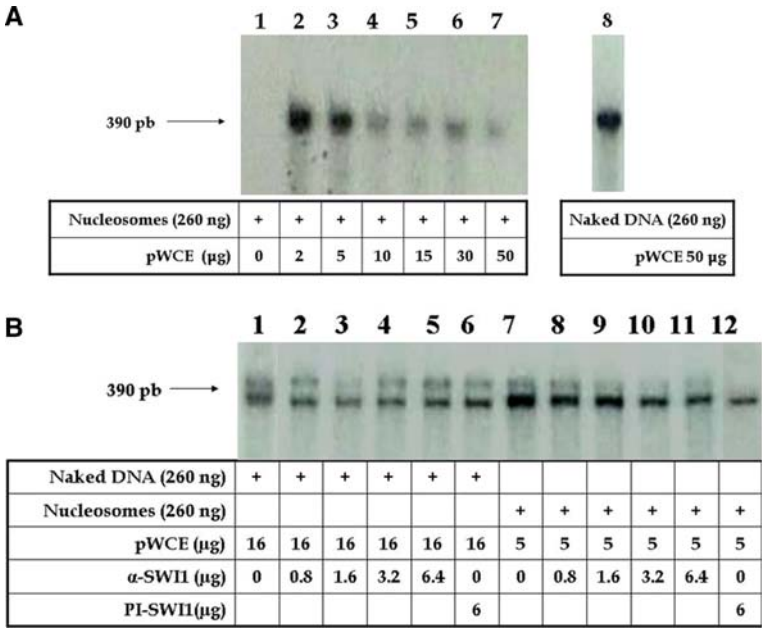

Fig. 7 (**A**) Effect of the *Sc. pombe* concentration extract (pWCE) over the transcriptional activity on nucleosomal arrays. Different concentrations of the cellular extract were assayed (Lanes 1–6) and the transcriptional activity was measured with the apparition of a 390 bp transcript. The control was naked DNA, with a high cellular extract concentration (Lane 7). (**B**) Effect of the α-Swi1 antibodies on the transcription process. A 390 bp transcript is observed when naked DNA was used (Lanes 1–6) or nucleosomal DNA (Lanes 7–12). In each, growing concentrations of α-Swi1 antibody were assayed. Pre-immune serum was used as control (Lanes 6 and 12)

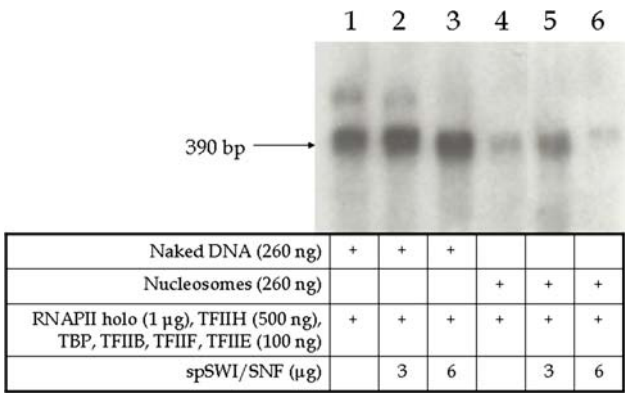

Fig. 8 Effect of the fraction containing the spSWI/SNF complex on a reconstituted transcriptional assay. Two concentrations of the spSWI/SNF complex were used on naked DNA (Lanes 2 and 3) and on nucleosomal DNA arrays (Lanes 5 and 6). As a control reconstituted transcriptional assays were performed in absence of the complex (Lanes 1 and 4)

Purification of the spSWI/SNF complex was done by conventional chromatography from a cellular extract of *Sc. pombe*, and the elution was monitored with antibodies against the spSwi1-like subunit.

A different protein of *Sc. pombe*, named Swi1, has been reported previously, but is a component of a replication fork protection complex [26, 27], and has no homology with *S. cerevisiae* Swi1. Thus, we named our protein spSwi1-like.

The Western Blot assays against spSwi1-like or spBrg1 proteins showed discrete bands, probably due to the low concentration of spSWI/SNF complex in the *Sc. pombe* extract. This has been observed in *S. cerevisiae*, where the concentration of SWI/SNF complex is 10-fold lower than RSC, another chromatin remodeling complex [13, 14, 28]. Moreover, according to our results, higher concentrations of cellular extract of *Sc. pombe* (50 µg), commonly used in vitro transcriptional assays with naked DNA [21, 29], inhibit the process when nucleosomal arrays of DNA are used. This inhibition is probably due to histone free ends on nucleosomes interacting with different transcription factors, both inhibitors and activators, which do not occur with naked DNA. Complexes like HDACs, probably activate with higher *Sc. pombe* extract concentrations, diminishing transcriptional activity. Nevertheless, optimal transcriptional assays in nucleosomal arrays have been reported with high concentrations of HeLa cells extract (80 µg) [20].

In our transcriptional assays with *Sc. pombe* cellular extracts, the addition of Swi1 antibodies to the reaction had no effect, indicating that other complexes like RSC, ISWI, Ino80, or Mi2 exist in the extract, which could remodel the structure of chromatin, replacing the activity of spSWI/SNF. According to these results, a homolog to Sth1, the catalytic subunit of *S. cerevisiae* RSC, exists in *Sc. pombe*, which possesses 1,199 amino acids and a 48% of amino acid sequence identity with its homolog. In contrast to ySWI/SNF, the RSC function is required for yeast viability [14, 25]. Moreover, genome-wide gene expression studies have revealed that ySWI/SNF and RSC regulate different non-overlapping sets of target genes [14].

We described a reconstituted transcriptional assay with recombinant factors TBP, TFIIB, TFIIF, and TFIIE, and the highly purified TFIIH and RNAPII holoenzyme complexes. These factors are required for basal transcription on

naked DNA, but they are insufficient when nucleosomes are used, indicating that chromatin remodeling is needed. Furthermore, we also studied the effect of spSWI/SNF in a reconstituted transcriptional assay. We found that when the concentration of this complex was adequate (3 μg), it promoted transcription, but when larger concentrations were used (6 μg), transcription was inhibited. This could be due to an unspecific interaction of the spSWI/SNF complex with other factors present in the basal machinery of transcription. It has long been known that the SWI/SNF complex can cooperate with many transcriptional factors to modulate transcription [30, 31], which concurs with the present findings. The SWI/SNF complexes act at very low concentrations, where one molecule is able to modify multiple nucleosomes on an assembled plasmid [32]. We can also explain that in the fraction containing the spSWI/SNF complex, molecules that inhibit the transcriptional process may be present, as observed with the whole extract. The control was naked DNA and no effect was observed when different concentrations of spSWI/SNF were added, which indicates that the interaction is mainly with histones. It has been demonstrated that several ATP-dependent nucleosome remodeling complexes can also either remove or exchange histone dimers [33].

When we immunoprecipitate the spSWI/SNF complex, we observed that almost seven subunits were present, among which spBrg1 (148 kDa) and spSwi1-like (96 kDa) were identified by Western Blot. SWI/SNF possesses 11 subunits in *S. cerevisiae*, eight subunits in *Drosophila* BAP and nine in human BAF [14].

Finally, we concluded that *Sc. pombe* possesses a complex belonging to the SWI/SNF family, like many other organisms, and this promotes transcription in a reconstituted assay with nucleosomes assembled in vitro, and recombinant and highly purified factors.

This is the first time that a remodeling complex has been purified from *Sc. pombe*, but it is still necessary to find more complexes able to modify the chromatin structure because new relations with cancer, embryonic development, cellular cycle, and DNA-repair are being found.

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