SYT Associates with Human SNF/SWI Complexes and the C-terminal Region of Its Fusion Partner SSX1 Targets Histones*

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A global transcriptional co-activator, the SNF/SWI complex, has been characterized as a chromatin remodeling factor that enhances accessibility of the transcriptional machinery to DNA within a repressive chromatin structure. On the other hand, mutations in some human SNF/SWI complex components have been linked to tumor formation. We show here that SYT, a partner protein generating the synovial sarcoma fusion protein SYT-SSX, associates with native human SNF/SWI complexes. The SYT protein has a unique QPGY domain, which is also present in the largest subunits, p250 and the newly identified homolog p250R, of the corresponding SNF/SWI complexes. The C-terminal region (amino acids 310–387) of SSX1, comprising the SSX1 portion of the SYT-SSX fusion protein, binds strongly to core histones and oligonucleosomes in vitro and directs nuclear localization of a green fluorescence protein fusion protein. Experiments with serial C-terminal deletion mutants of SSX1 indicate that these properties map to a common region and also correlate with the previously demonstrated anchorage-independent colony formation activity of SYT-SSX in Rat 3Y1 cells. These data suggest that SYT-SSX interferes with the function of either the SNF/SWI complexes or another SYT-interacting co-activator, p300, by changing their targeted localization or by directly inhibiting their chromatin remodeling activities.

The chromatin structure of active eukaryotic genes is subject to dynamic change by chromatin modifiers such as ATP-dependent chromatin remodeling factors (reviewed in Refs. 1–5). Homologs of a yeast prototype ATP-dependent remodeling complex, SNF/SWI, appear to be widely present in eukaryotes from yeast to humans (6–8). Functions of the subunits of the SNF/SWI complexes (9, 10) were first demonstrated by genetic studies in Saccharomyces cerevisiae, which showed that SWI1/
the c-fos gene (48). Moreover, phosphatidylinositol 4,5-biphosphate, a major signal mediator in lymphocytes, induces translocation of SNF/SWI complexes to chromatin (49). Finally, involvement of the SWI/SNF proteins in malignant transformation has been highlighted by the discovery that the INI1/hSNF5 gene is frequently mutated in rhabdoid tumors (50). A number of studies have reported alterations of the genes encoding SNF/SWI subunits in various human cancers (51, 52).

Synovial sarcomas are typified by a unique chromosomal translocation t(X;18)(p11.2;q11.2) that results in fusion of the SYT gene on chromosome 18 with the SSX1 or SSX2 gene in Xp11.2 and, consequently, production of the chimeric SYT-SSX proteins (53). SYT is a ubiquitously expressed protein with a QGY domain, whereas the SSX proteins carry KRAB-like domains and are expressed almost exclusively in the testis (54). The SYT and SSX proteins localize in distinct nuclear domains. Localization of the SYT-SSX fusion proteins does not appear to be completely identical to that of either SYT or SSX, although variability has been reported (55–58). Interestingly, the SYT-SSX and SYT proteins appear to localize in particular nuclear speckles where hbrm protein is present (59). Thus, altered localization of the SYT-SSX proteins and their associated proteins may account for the underlying mechanisms of synovial sarcoma formation.

Here we report that the SYT protein in fact is present in native human SNF/SWI complexes and shares the QGY domain with the largest subunits of these complexes. Interestingly, the C-terminal 78-amino acid region of SSX1 binds strongly to core histones and oligonucleosomes. Deletion analysis reveals that this activity correlates both with the nuclear localization of the SSX1 C-terminal domain and with the transactivation activity of SYT-SSX in rat 3Y1 cells (60). We hypothesize that the forced mislocalization or dysfunction of the SYT-associated chromatin remodeling factors, such as the SNF/SWI complexes or the SYT-interacting co-activator p300, by SYT-SSX is the cause of the malignant transformation.

**EXPERIMENTAL PROCEDURES**

**Purification of Human SNF/SWI Complexes—**Human SNF/SWI complexes were affinity-purified on anti-FLAG antibody (M2)-conjugated agarose (Kodak/IBI) from nuclear extracts prepared from FLAG-tagged INI1-expressing HeLa (F-Ini1/HeLa) cells essentially as described by Sif et al. (44). Complexes were washed extensively (five times) with buffer D (20 mM Tris-HCl, pH 7.9, 10% glycerol, 1 mM dithiothreitol (DTT), 0.2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 0.1% Nonidet P-40) containing 500 mM KCl and eluted with an acidic solution. Antisera to SYT (C44) and SSX2 (B39), generously provided by D. de Bruijn, were used for coupling on Protein G-Sepharose 4F (Amersham Biosciences, Inc.) with dimethyl pimelimidate dihydrochloride (Sigma Chemical Co.).

**Protein Identification by Mass Spectrometry—**The SNF/SWI complexes were separated by 4 to 20% gradient SDS-PAGE and visualized by staining with Coomassie Brilliant Blue (CBB) R-250 or by negative staining with zinc sulfate and imidazole (Bio-Rad). The excised bands were destained and processed by in-gel digestion with trypsin (61). Molecular masses of the peptides were determined by MS analysis with matrix-assisted laser desorption/ionization quadrupole-time-of-flight mass spectrometry (61), and the assignment was confirmed by the subsequent MS/MS analysis of selected ion species. Proteins were identified by using the search engines ProFound (62) and PepFrag (63).

**Molecular Cloning of the p250 and p250R cDNAs—**Terminal and N-terminal B120 fragments (64) were used to screen a human fetal brain cDNA library in AZAP II (Stratagene) for p250 cDNAs. The most abundant spliced variant in the 5′-end sequence was obtained from HeLa cDNA by using the oligo-capping cloning method as described previously (65). Briefly, the PCR template was synthesized with reverse transcriptase and an oligo-dT primer from HeLa cDNA 5′-capped with an oligo RNA (5′-AGCAUCAGAUCCGCUUGUUGGCCCUACUC-3′). A nested PCR reaction was performed with cap primers (5′-AGCATCGAGCTGCTTGGCTG-3′ and 5′-GAGTGGCCTTGGCTGACTTC-3′) and p250R internal primers corresponding to nt 2024–2001 and 1925–1902. The p250R cDNA is a composite of HeLa (nt 1–2590) and the fetal brain (nt 2591–5123) cDNAs.

**Immunoblot Analysis—**Affinity-purified or corresponding GST fusion proteins coupled to CNBr-activated Sepharose 4B (Amersham Biosciences, Inc.) were cross-linked to CNBr-activated Sepharose 4B and used for purification of the SNF/SWI complexes from HeLa nuclear extract. Antibody coupling and subsequent washing of the cross-linked matrix were performed under conditions recommended by the manufacturer. Immune complexes were washed extensively (four to five times) with buffer D containing 500 mM KCl and eluted with an acidic solution. Antisera to SYT (C44) and SSX2 (B39), generously provided by D. de Bruijn, were used for coupling on Protein G-Sepharose 4F (Amersham Biosciences, Inc.) with dimethyl pimelimidate dihydrochloride (Sigma Chemical Co.) at a final concentration of 60 μg/ml for 30 min at room temperature. Monoclonal antibody to FLAG epitope (M2) and M2 conjugated to Sepharose were purchased from Sigma.

**Immunoblot Analysis—**Affinity-purified or extracts were resolved by SDS-polyacrylamide gel electrophoresis, transferred onto a nitrocellulose membrane (Hybond-ECL, Amersham Biosciences, Inc.), incubated first with an antiseraum or a purified antibody and then with a horseradish peroxidase-conjugated secondary antibody (anti-rabbit for all polyclonal antibodies or anti-mouse for anti-FLAG antibody), and detected by chemiluminescence (ECL detection reagents, Amersham Biosciences, Inc.). Antisera and the M2 antibody were used in 1000-fold dilution and at a final concentration of 4 μg/ml, respectively.

**GST Pull-down Assay—**All of the GST fusion proteins were expressed in BL21(DE3)pLysS (Novagen), and extracts were prepared by sonication in lysis buffer (20 mM Tris-HCl (pH 7.9), 300 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.1% Nonidet P-40, 1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml aprotinin, and 1 μg/ml pepstatin A). Native core histones and oligonucleosomes were purified from HeLa cells as described (66). Recombinant full-length and tailless histones were expressed in Escherichia coli and purified. GST proteins (~3–6 μg) bound to glutathione-Sepharose beads were incubated with HeLa nuclear extract (5 mg) or recombinant H2A, H2B, H3, and H4 histones (~0.5 μg each) and

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W. An and R. G. Roeder, unpublished data.
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RESULTS

SYT Protein Is Present in Native SNF/SWI Complexes—Thaete et al. (59) reported co-localization of SYT with hbrm in characteristic nuclear speckles. However, the presence of endogenous SYT protein in native SNF/SWI complexes has not been demonstrated. To investigate this possibility, the SNF/SWI complexes (Fig. 1) purified from FLAG-tagged Ini1-expressing HeLa cell nuclear extract (44) by an immunoprecipitation method using anti-FLAG monoclonal antibody (M2)-conjugated agarose beads were analyzed by Western blotting. An anti-SYT serum (C44) detected two strong bands of 53 and 65 kDa in F-Ini1-HeLa nuclear extract and in all three preparations of the purified SNF/SWI complexes but not in the control SPT5 complex isolated from a FLAG-tagged SPT5-expressing HeLa cell nuclear extract (44m). The observed and calculated m/z values of the fragment ions from the candidate peptide (Fig. 2B, lane 1) was at least as efficient as the anti-Ini1 serum (lane 4) in immunoprecipitating F-Ini1; however, two other control antisera (anti-SSX2 and anti-p73L) were not able to significantly precipitate FLAG-Ini1 protein (lanes 2 and 3). To investigate the abundance of the SYT-containing SNF/SWI complexes in the F-Ini1-containing SNF/SWI complexes, anti-SYT and anti-FLAG immunoprecipitates were analyzed by Western blot for relative amounts of SYT and F-Ini1 between the purified SNF/SWI complexes and the F-Ini1-HeLa nuclear extract are equivalent (Fig. 2A).

To further demonstrate an association between SYT and SNF/SWI complexes, we tested whether the anti-SYT serum immunoprecipitates F-Ini1 protein in the extract. An anti-SYT antibody covalently cross-linked to protein G-Sepharose was incubated with F-Ini1-HeLa nuclear extract, and the resulting pull-down protein was analyzed by immunoblotting with anti-FLAG M2 monoclonal antibody. The anti-SYT serum clearly precipitated F-Ini1 (Fig. 2B, lane 1) was at least as efficient as the anti-Ini1 serum (lane 4) in immunoprecipitating F-Ini1; however, two other control antisera (anti-SSX2 and anti-p73L) were not able to significantly precipitate FLAG-Ini1 protein (lanes 2 and 3). To investigate the abundance of the SYT-containing SNF/SWI complexes in the F-Ini1-containing SNF/SWI complexes, anti-SYT and anti-FLAG immunoprecipitates were analyzed by Western blot for relative amounts of SYT and F-Ini1. The percentage of SYT-containing SNF/SWI complexes was estimated as at least 3.4% of the total F-Ini1-containing SNF/SWI complexes (data not shown).

To rule out the possibility that the signals were due to cross-reaction of the anti-SYT serum with some of the enriched as described under “Experimental Procedures.” Trypsin-digested protein samples were analyzed by mass spectrometry. A candidate peptide from the 53- to 55-kDa sample matching the C-terminal peptide of the SYT protein was further analyzed by the MS/MS spectra (61). The observed and calculated m/z values of the fragment ions from the candidate peptide are shown.
SNF/SWI components, we performed a mass spectrometric analysis of tryptic peptides obtained from several SDS-polyacrylamide gel slices in the 53–55 kDa range (Fig. 1). In one of these slices, we detected a peak at m/z 1680.68 that exactly matches the calculated m/z value for the SYT peptide PYGY-DQQGYGNYQQ. Subsequent MS/MS spectrometric analysis of the m/z 1680.68 peptide confirmed this identification (Fig. 2C).

From the relatively low observed signal intensities, we suspect that SYT is a sub-stoichiometric component of the complex. This is the first demonstration of SYT association with native SNF/SWI complexes.

**p250 and a Related Protein, p250R, Are Intrinsic Components of Human SNF/SWI Complexes**—In a separate experiment, we analyzed the approximate 50-kDa bands in the SNF/SWI complexes and noticed that a short amino acid motif is commonly present in both SYT and 250-kDa proteins. We therefore became interested in the relationships between these proteins with respect to structure and complex formation. Mass spectrometric analyses of the 250-kDa protein isolated from the SWI/SNF complexes (Fig. 1) revealed that a number of derived peptide sequences matched those in the previously described B120 protein (64). Peptide sequences attributed to p250 correspond to amino acids 236–732, 821–862, 877–912, 902–915, 975–1009, 1010–1022, 1141–1150, 1243–1251, and 1804–1822. Notably, TPQPSSPMQMGK (amino acids 236–261) enabled us to distinguish a particular cDNA encoding this sequence from cDNAs encoding a number of splice variants. Screening of a phage cDNA library led to isolation of a cDNA encoding a 1939-amino acid protein, referred to as p250, with an N-terminal half that is almost identical to B120 and a novel C-terminal half. A noted difference between the N termini of p250 and B120 is due to splicing variation. At least several additional splice variants with the same acceptor site have been found (data not shown). Cloning of cDNAs corresponding to p250 (designated p270 or BAF250) has been recently reported by others, and further suggests that p250 has additional N-terminal amino acids (67, 68). By searching the GenBank™ expressed sequence tag sequences, we found expressed sequence tag sequences with close similarity to p250 cDNA. Screening of a phage cDNA library for p250 cDNA and identification of the 5′ end by the oligo-capping cloning method (65) revealed the presence of a close homolog of p250, referred to as p250R, that is composed of 1486 amino acids and shows strong identity to p250 throughout the entire coding sequence (Fig. 3). p250R lacks a region corresponding to the p250 N terminus. Because the initiator ATG is preceded by a termination codon in the p250R 5′-terminal cDNA obtained by the oligo-capping method, this ATG may be the bona fide initiator. Alternatively, other potential splice variants may have a further N-terminal sequence. The p250 protein shows strong sequence homology to the Drosophila Osa/eyelid protein. Osa/eyelid, originally identified as a trithorax group member (69), has organ-specific genetic interactions with brm, mor, and snr1 (homologs of SNF2/SWI2, SWI3, and SNF5, respectively) and antagonizes wingless signaling (70, 71). Both of the p250 and p250R proteins have a highly conserved region, referred to as the ARID domain (72), that has been found in more than a dozen other proteins (Fig. 3). These include putative matrix-associated region-binding proteins, the Drosophila Dead ringer (73) and Bright (74) proteins and the putative homologs of p250 and p250R, Osa/eyelid and yeast SWI1. Another remarkable feature also common to p250 and p250R is the presence of a number of peptide motifs containing tyrosine residues surrounded by several proline (P), glutamine (Q), and glycine (G) residues (Fig. 3). Similar QPGY motifs, comprising a QPGY domain, have been reported for the SYT protein (59). The SYT-SSX fusion proteins found in synovial sarcomas (53) are typically composed of the first 379 amino acids of SYT (excluding only the C-terminal 8 amino acids) and the C-terminal 78 amino acids of SSX (SSXC). The SYT-SSX proteins retain most of the QPGY motifs. This motif is tentatively defined in this report as XXXYX, where a tyrosine residue is surrounded by at least three X residues are either P, Q, or G. p250, p250R, and SYT have 31, 20, and 18 motifs, respectively (Fig. 3).

We next analyzed p250- and p250R-containing complexes by immunoprecipitation with specific affinity-purified antibodies. Both anti-p250 and anti-p250R antibodies precipitated complexes with a subunit composition similar to those of F-Ini1-containing complexes (Fig. 4A, lanes 5 and 6). However, the largest subunits, presumed to be p250 and p250R, were found to be somewhat different, because the latter migrated as a broad band. When these samples were separated by 5% SDS-PAGE and analyzed by Western blotting with the anti-p250R antibody, p250 appeared as a sharp 250-kDa band and p250R appeared as two broad bands around 260 and 220 kDa (Fig. 4B). These results indicate that the two antibodies specifically...
precipitated different complexes containing corresponding p250 and p250R antigens without cross-reaction. After being denatured, however, both antigens are recognized by the anti-p250R antibody, probably because of the similarity between p250 and p250R (43% identical in the corresponding region). Similarly, anti-p250 antibody also detected these bands (data not shown). Thus we conclude that p250 and p250R are intrinsic and mutually exclusive components of human SNF/SWI complexes.

**The C-terminal Region of SSX1 Binds to Core Histones**—The observation that SYT is associated with the native SNF/SWI complexes led us to investigate the molecular mechanisms that enable SYT-SSX proteins to eventually cause malignant tumors. We hypothesized that the SSX C-terminal domain provides that property by interacting with protein targets. To address this question, we used an affinity-purification method involving GST pull-downs. Sepharose beads containing GST fusion proteins were incubated with HeLa nuclear extract, washed, and eluted. Associated proteins were analyzed on a 10% polyacrylamide-SDS gel (Fig. 5A). Proteins pulled down by a fusion protein containing the 78-amino acid C-terminal region of SSX1 (SSX1C) showed specific bands of low molecular masses (<20 kDa) and two specific bands of high molecular masses (70–90 kDa) (lane 4). Because the low molecular mass proteins have a pattern similar to core histones, they were compared with purified native core histones in a side-by-side manner (Fig. 5B). Both the pulled-down proteins and the core histones showed indistinguishable sets of four bands (corresponding to H3, H2B, H2A, and H4 from upper to lower) on 4 to 20% gradient polyacrylamide-SDS gel (compare lane 1 with lane 2). A mass spectrometric analysis revealed that two high molecular mass bands are identical to Ku70 and Ku80 (data not shown). We suspect that Ku70 and Ku80 were precipitated through binding to DNA ends of nucleosomes, because it is likely that at least some fractions of the core histones present in the nuclear extract exist as oligonucleosomes.

To know whether SSX1C binds directly to core histones and to determine the region in SSX1C required for this binding, a set of C-terminally truncated SSX1C mutants were tested for interaction with purified DNA-free core histones (Fig. 5C). Most of the core histones were retained on GST-SSX1C (compare B with UB in GST-SSX1C). A somewhat smaller fraction of histones was co-precipitated with the 11-amino acid truncation mutant (GST-SSX1Cd11). However, a further deletion that removes the C-terminal 34 amino acids severely affected, but did not completely abolish, the binding (compare UB with B in GST-SSX1CdRD). These results indicate that the C-terminal SSX1 domain, the SSX part of the SYT-SSX1 fusion protein, in fact binds to core histones and that the interaction

HeLa nuclear extract (lanes 3 and 4). The pull-down fractions were eluted with buffer D containing 0.1% sodium deoxycholate and analyzed by 10% SDS-PAGE and staining with CBB R-250. Specific low molecular weight proteins are marked by the vertical line. Two specific high molecular weight bands are indicated by arrowheads. B, side-by-side comparison with the native core histones. The same sample as in lane 4 of panel A (lane 1) was separated by 4 to 20% gradient SDS-PAGE with the core histones (lane 2) purified from HeLa cells. C, GST-SSX1C mutants with serial deletions were tested for binding to the purified core histones. GST-SSX1Cd11 and GST-SSX1CdRD have 11- and 34-amino acid deletions from the SSX1 C terminus, respectively. For quantitative comparison, bound fractions were directly suspended in SDS sample buffer and analyzed by 4 to 20% SDS-PAGE. IH, IR, UB, and B denote input core histones, input recombinant GST fusion protein, unbound fraction, and bound fraction, respectively. Arrowheads indicate GST fusion proteins. D, binding to purified oligonucleosomes was analyzed as in C. E, binding to tailless histones. Full-length and tailless recombinant core histones were used for the pull-down assay. Arrowheads indicate GST fusion proteins.
Numerous ATP-dependent chromatin remodeling complexes have been reported and shown to possess similar chromatin-disruption activities (24, 25, 76–82). Genetic studies in yeast have revealed that functions of the remodeling factors are likely to be distinct in some cases and partially redundant in others (77, 82). However, molecular mechanisms to explain the apparent differences in mutant phenotypes have not been well elucidated. A possible explanation is that these complexes physically and functionally interact with different regulatory molecules and/or are recruited to different chromosomal target sites by protein-protein interactions. In the case of mammals, determination of the primary structures of the subunits, identification of associated proteins, and findings of connections with phenotypes of genetic diseases or of mutants generated by genetic engineering have helped predict their physiological function (7). In this context, further identification and characterization of the stoichiometric components and the associated proteins could provide insights into SNF/SWI function.

**SYT Is an Intrinsic Component of SNF/SWI Complexes**—A remarkable feature of the p250 and p250R proteins is the presence of numerous QPGY motifs, comprising a QPGY domain, as has been reported for the SYT protein (Fig. 3). The QPGY domain is not evident in other proteins in the data base (data not shown). Because the SYT QPGY domain has a transcription-activation activity (59), these QPGY domains may serve as interaction interfaces with common components such as one of the SNF/SWI subunits or other transactivators. However, this possibility has not been addressed in this article. Motif sharing between p250/p250R and SYT is intriguing in light of the co-localization of SYT with hBRM in characteristic nuclear speckles (59). We have shown here that SYT is present in at least a subpopulation of purified native SNF/SWI complexes by Western blot and mass spectrometry analyses (Fig. 2, A–C).

Nagai et al. (60) recently reported that rat fibroblast 3Y1 cells expressing SYT-SSX1, but neither SYT nor SSX1 alone, exhibit increased growth rate, anchorage-independent growth in soft agar, and tumor formation in nude mice, presumably through interaction with hBRM/sSNF2a. Consistent with this observation, simple C-terminal truncation mutants of SYT in synovial sarcomas have not been reported so far. These data strongly suggest that the SSX C-terminal region confers a novel ability to cause malignant transformation on SYT. Very interestingly, Eid et al. (83) recently showed that SYT associates with the histone acetyltransferase p300 and promotes cell adhesion to a fibronectin matrix, although the effect of SYT-SSX on p300 has not been investigated. A recent study has suggested that an ATP-dependent chromatin remodeling factor and a histone acetyltransferase activate the chromatin template in a sequential manner (84). Taken together, one could anticipate that SYT plays a common role in the function of both SNF/SWI complexes and p300 and that SYT fused with the SSX C terminus interferes with one or more steps of chromatin activation achieved by these apparatuses (84). We therefore sought to identify target proteins (most likely interacting proteins) of the SSX C-terminal region.

**Presence of Multiple SNF/SWI Complexes Containing Alternative Large Subunits**—We have characterized a large subunit of the mammalian SNF/SWI complexes, p250, and a closely related protein, p250R. Both p250 and p250R are intrinsic and stoichiometric components of corresponding complexes. These proteins share a highly conserved ARID domain. Recently, other groups also reported the isolation and characterization of p250 (p270 or BAF250) and showed that the ARID domain of p250 exhibits nonspecific or pyrimidine-preferred DNA binding activity (67, 68). We have also failed to detect clear sequence-
specific DNA-binding activity with GST-p250 and GST-p250R proteins in vitro (data not shown). Collins et al. (71) showed that the Drosophila Osa protein (potential homolog of p250/p250R) also has nonspecific DNA-binding activity and distributes over the entire length of all the polytene chromosomes. Nevertheless, judging from an experiment showing that ectopic expression of an Osa ARID domain-VP16 activation domain fusion protein provides some Osa function, it appears that the Osa ARID domain has functional specificity (i.e. targeting specific promoters) (71). These results, combined with the fact that the ARID domain is the only sequence apparently conserved between SWI1 and Osa or p250/p250R, suggests that the ARID domain may have unidentified target-recognition activity.

The subunit compositions of the complexes isolated via antibodies directed against p250 and p250R are indistinguishable except for the p250 and p250R bands (Fig. 4A). The p250-containing complex has a clear stoichiometric band of 250 kDa, whereas the p250R-containing complex has broad bands of 220 and 260 kDa. The SNF/SWI complexes were originally reported to be chromatographically separable into complexes A and B (24). A recent report showed that complex A (BAF) contains p250 (BAF250), whereas complex B (PBAF) contains a 180-kDa protein (BAF180) that is unrelated to p250 in primary structure and carries multiple bromodomains and two BAH regions (82). The authors suggested that PBAF is slightly different from the originally reported complex B and more closely related to the yeast RSC complex than to the yeast SNF/SWI complex. It is possible that the p250R-containing complex could represent the original complex B or that unseparated p250- and p250R-containing complexes could represent complex A. Our data thus extend structural, and potentially functional, complexity to the SNF/SWI complexes. Interestingly, the Drosophila SNF/SWI complexes may also have multiple alternative subunits to Osa, because the complexes precipitated by α-Bram contain additional high molecular weight bands other than those corresponding to Osa (71). The presence of multiple alternative subunits and the observed transcriptional regulation by p250/BAF250 (68) and Osa (71) imply that these subunits somehow regulate the SNF/SWI activities and that they may interfere with one another.

An interesting possibility is that SYT is physically or functionally related to p250 and p250R. A Western blot analysis showed that p250- and p250R-containing complexes clearly contain SYT, suggesting that SYT is not an alternative subunit to p250 or p250R (data not shown). Eid et al. (83) reported that, in contact-inhibited or adhesion cells, SYT becomes tyrosine-phosphorylated, possibly in the QPGY domain, and forms complexes with p300 and at least two other proteins (83). The QPGY domains of p250 and p250R may also serve as residues for tyrosine phosphorylation and protein-protein interactions.

Correlation between Histone Binding and Nuclear Localization—Our finding, based on deletion studies, that the histone-binding activity of SSX1C parallels its nuclear localization activity could be related to the previous observations that the SSX1 and SSX2 proteins localize in the nucleus and associate with mitotic chromosomes (56–58). It is thus likely that SSX proteins associate with chromatin throughout the cell cycle via direct binding of SSXC to core histones. Here it is noteworthy that an SYT-SSX1 protein, with the same truncation of the SSX1 C-terminal 34 amino acids as SSX1C4RD, exhibited weaker yet still significant anchorage-independent colony formation activity in soft agar (60). Thus it appears that binding to core histones, nuclear localization, and anchorage-independent growth all approximately correlate with one another, indicating the possibility that the histone-binding and nuclear localization activities contribute to malignant transformation.

Possible Molecular Mechanisms of Malignant Transformation by the SYT-SSX Fusion Protein—These results, in conjunction with the observation that several of the SNF/SWI components are potentially negative growth regulators or tumor suppressors (50, 51, 86, 87), have led us to propose the following working hypotheses to explain the molecular mechanisms of the SYT-SSX action in tumor formation (Fig. 7). First, the histone-binding activity of SSX1C directly inhibits the SNF/SWI remodeling activity by stabilizing or changing the nucleosome structure or, alternatively, by blocking the interaction between the SNF/SWI complexes and the core histones (Fig. 7A). This model may also explain mechanisms of potential interference with p300 activity by SYT-SSX (23). Second, SSX1C recruits the SNF/SWI complexes to aberrant target sites by interacting with modified chromatin (e.g. containing acetylated or methylated histones or a specific configuration) (Fig. 7B). The fact that the SSX1C-histone interaction requires the N-terminal tails of the core histones for maximum affinity suggests that the interaction may be influenced by histone modifications. Because bromodomains of several nuclear proteins specifically interact with a histone H3 or H4 tail in an acetylation-dependent manner (reviewed in Ref. 88), the association of SNF/SWI complexes and p300 (both hbrm/BRG-1 and p300 are bromodomain-containing proteins) with SYT-SSX may alter target specificity. Third, SYT-SSX1C sequesters the SNF/SWI complexes from the regular target site to other sites by forced binding (Fig. 7C). Because the SYT-SNF/SWI complexes appear to be a minor population among the total SNF/SWI complexes, sequestration by SYT-SSX would be from SYT-specific target sites. These possibilities are currently being investigated.

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