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The C-terminal SET domains of ALL-1 and TRITHORAX interact with the INI1 and SNR1 proteins, components of the SWI/SNF complex

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ABSTRACT **The *ALL-1* gene was discovered by virtue of its involvement in human acute leukemia. Its *Drosophila* homolog *trithorax* (*trx*) is a member of the *trx-Polycomb* gene family, which maintains correct spatial expression of the *Antennapedia* and bithorax complexes during embryogenesis. The C-terminal SET domain of ALL-1 and TRITHORAX (TRX) is a 150-aa motif, highly conserved during evolution. We performed yeast two hybrid screening of *Drosophila* cDNA library and detected interaction between a TRX polypeptide spanning SET and the SNR1 protein. SNR1 is a product of *snr*1, which is classified as a *trx* group gene. We found parallel interaction in yeast between the SET domain of ALL-1 and the human homolog of SNR1, INI1 (hSNF5). These results were confirmed by *in vitro* binding studies and by demonstrating coimmunoprecipitation of the proteins from cultured cells and/or transgenic flies. Epitope-tagged SNR1 was detected at discrete sites on larval salivary gland polytene chromosomes, and these sites colocalized with around one-half of TRX binding sites. Because SNR1 and INI1 are constituents of the SWI/SNF complex, which acts to remodel chromatin and consequently to activate transcription, the interactions we observed suggest a mechanism by which the SWI/SNF complex is recruited to ALL-1/*trx* targets through physical interactions between the C-terminal domains of ALL-1 and TRX and INI1/SNR1.**

The *ALL-1* gene is involved in human acute leukemia, in particular infant and secondary leukemia, through chromosome translocations or partial tandem duplications (1–3). The chromosome translocations result in expression of chimeric proteins composed of the N-terminal ≈1,300 residues of ALL-1 linked to a C-terminal polypeptide encoded by any (>25) of the partner genes. The gene duplications produce altered (longer) ALL-1 proteins. ALL-1 is the human homolog of *Drosophila trx* (4). The latter is a member of the *trithorax* group (*trx-G*) gene family that together with the *Polycomb* group (*Pc-G*) genes act to control expression of the *Antennapedia* and bithorax homeotic gene complexes determining segment identify in *Drosophila* (ref. 5 and reviewed in refs. 6 and 7). Expression or silencing of the homeotic genes (HOM) is established by the gap and pair-rule genes at the cellular blastoderm stage. This determined state is maintained during subsequent development by the collective action of the *trx-G* and *Pc-G* genes, which function as transcriptional activators and repressors, respectively. *trx-G*/*Pc-G* genes act through specific response elements present within their target genes (8, 9). This activity is evidenced by the physical localization of the protein products of several *trx-G*/*Pc-G* genes, including *trx* and *Pc*, to multiple sites (presumed to be the response elements in target genes) on salivary gland polytene chromosomes of third star larvae (refs. 10–13 and references therein).

Converging lines of evidence suggest that *trx-G* and *Pc-G* genes act by establishing open and repressive chromatin states, respectively. A striking feature is the similarity in structure (14) and function (15, 16) between some *trx-G*/*Pc-G* proteins and suppressors or enhancers of variegation. The latter are genes that affect transcription of genes embedded within heterochromatin. More direct evidence emerged from the identification of three *trx-G* proteins as components of multiprotein complexes which open (remodel) chromatin and consequently facilitate transcription. Thus, the GAGA protein, which is the product of the *trx*-like gene, was demonstrated to participate together with the NURF complex in nucleosome unfolding (17). Similarly, the *Drosophila brm* and *snr1* genes were found to be the homologs of two yeast genes (*Swi2* and *snf5*, respectively), which products are components of the chromatin-remodeling complex SWI/SNF (for reviews, see refs. 18 and 19). A similar complex exists in *Drosophila* (20) and was purified to homogeneity from mammalian cells (21). The failure to detect sufficient sequence specificity in binding of SWI/SNF to DNA (18) has raised the question as to the mechanism by which the complex is recruited to target genes. We propose here that ALL-1/*trx* is involved in this process.

MATERIALS AND METHODS

Yeast Two Hybrid Screening. The HF7c and SFY526 yeast strains as well as the pGBT9 vector were obtained from Clontech (Matchmaker two hybrid system). Screening and verification of the specificity of the interactions were done according to manufacturers' instructions. The analysis included growth of HF7c transformants on His^- plates containing 5–20 mM 3-aminotriazol and synthesis of β-galactosidase in SFY526 and HF7c transformants.

Construction of Transgenic Lines with Epitope-Tagged SNR1. A cDNA fragment containing the full-length ORF of *snr1* was tagged at the N terminus with the hemagglutinin (HA) epitope. The fragment was subcloned into the P-element transformation vector pCaSpeR-hs and injected into y^-w^- embryos. Transgenic lines homozygous for an insertion of transgene on the second chromosome were selected and used in immmunoprecipitation and rescue experiments. For rescue experiments, hsSNR1-HA transgenes were crossed into the

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Abbreviations: TRX, trithorax; PC, polycomb; HOM, homeotic genes; IP, immunoprecipitated; *trx-G*, *trithorax* group; *Pc-G*, *Polycomb* group; BRM, brahma; HA, hemagglutinin; hs, heat shock; E(Z), ENHANCER OF ZESTE; GST, glutathione S-transferase; Ab, antibody.

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background of the AS1319 *snr1* allele (20). Although at 20°C the AS1319 allele causes larval lethality (homozygotes die before the third instar larvae), we found that even a short treatment at 36°C during embryonic stages causes a complete embryonic lethality. Heat treatment of embryos homozygous for AS1319 and hsSNR1-HA transgene, at 36°C twice a day for 30 min during embryonic (5 and 10 hr after egg deposition) and again at larval stages, completely restored their viability at larval stages. Moreover, a substantial percent of the embryos survived until the third instar larval stage, suggesting that epitope-tagged SNR1 is functionally active.

***In Vitro* Binding.** TRX and ALL-1 C-terminal polypeptides were expressed as glutathione S-transferase (GST) fusion proteins in *Escherichia coli* and immobilized on affinity matrix beads. SNR1 and INI1 proteins were synthesized and radiolabeled in a coupled transcription-translation system (Promega kit). The labeled polypeptides were absorbed at 4°C for 2 hr to beads containing equal amounts of GST fusions or unlinked GST protein and then extensively washed. Binding and washing buffer contained 20 mM Tris (pH 8.0), 0.2% Triton X-100, 2 mM EDTA, 150 or 300 mM NaCl (for TRX and ALL-1 interactions, respectively) 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 1 μg/ml aprotinin. Bound proteins were eluted by boiling in 2X sample buffer and analyzed on a 10% SDS/PAGE gel.

Immunoprecipitation from *Drosophila* Extracts and Transfected Cells. Adult flies or 4- to 18-hr-old embryos were incubated for 1 hr at 36°C and followed by 30 min at 25°C and then collected. One hundred flies or 150–200 μl of dechorinated embryos were homogenized in 500 μl of a solution containing 50 mM Tris·HCl (pH 7.4), 250 mM NaCl, 5 mM EDTA, 0.1% NP40, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml aprotinin, and 1 μg/ml pepstatin and kept on ice for 30 min. After centrifugation at 14,000 rpm for 2 min, the supernatant was collected and diluted by adding 400 μl of similar buffer lacking NaCl. The solution was precleared by incubation with protein A-Sepharose beads for 40 min followed by centrifugation at 14,000 rpm for 5 min. The supernatant was mixed gently for 1 hr and subsequently incubated with affinity-purified anti-TRX Ab, preimmune serum, or affinity-purified Ab against the ENHANCER OF ZESTE (EZ) and polycomb (PC) proteins (obtained from R. Jones and P. O'Farrell, respectively) for 2 hr. Thirty microliters of protein A-Sepharose beads were added and incubation was continued for 1 h. Protein A-Sepharose beads were spun down and washed three times with 1 ml of binding buffer. Proteins were recovered by adding 30 μl of 2X sample buffer and boiling. SNR1 protein was detected by immunoblotting.

For transfection experiments, the different proteins were linked in their N-termini to T7 or HA epitopes, as well as to a nuclear localization signal. COS cells were transfected by the calcium phosphate technique with 10 μg of plasmid (pSG5 vector, Stratagene). At 48–72 hr post-transfection, the cells were washed with PBS, scraped and processed as above with the exception that protein G-Sepharose was used instead of protein A and preclearing was omitted. Five and two micrograms of anti-T7 (Novagen) and anti-HA mAb (Boehringer Mannheim), respectively were used for immunoprecipitation. For detection on blots, the anti-T7 and HA mAbs were diluted 1:10,000 and 1:250, respectively. For experiments involving endogenous ALL-1, 600 μg aliquots of cell nuclear extracts were diluted into 500 μl of binding buffer composed of 25 mM Tris (pH 7.8), 150 mM NaCl, 10% glycerol, 0.1 mM DTT, 0.2 mM each of EDTA and EGTA, 0. 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, and 10 μg/ml pepstatin A. The extracts were incubated at 4°C for 12 hr with affinity-purified ALL-1 Ab or other Ab and further incubated with protein G-Sepharose for 2 hr at 4°C with agitation. The precipitate was washed three times with binding buffer and one time with a solution of 0.1M Tris (pH 6.7), containing protease inhibitors. Pellet was resuspended in sample buffer; one-half of the eluate was analyzed on 5.5% SDS/PAGE to detect ALL-1, and the rest was analyzed on 10% gel to detect INI1.

Immunostaining of Polytene Chromosomes. Third star larvae of transgenic lines carrying hsSNR1-HA were collected 30 min after 1-hr incubation at 37°C and used to prepare salivary gland polytene chromosomes as described (12). Double-labeling of polytene chromosomes was carried out using anti-TRX polyclonal rabbit antibody (Ab) N1(12) at 1:50 dilution and anti-HA.11 mouse mAb against HA epitope tag (BabCO) diluted 1:100. Bound Ab were differentially detected with either Texas Red-conjugated goat anti-rabbit or with fluorescein isothiocyanate-conjugated goat anti-mouse (Jackson ImmunoResearch) secondary antibodies (both at 1:200 dilution). Vectashield (Vector Laboratories) was used as a mounting medium. Confocal image files of labeled chromosomes were obtained using a laser scanning confocal microscope and processed using the Adobe Photoshop program.

RESULTS AND DISCUSSION

While searching for proteins interacting with TRX and ALL-1 proteins, we chose the C-terminal region of the former as a bait for yeast two hybrid screening. The C-terminal region includes the SET domain, which is highly conserved between TRX and ALL-1 and also is present within the *trx-G/Pc*-G proteins ASH 1 (13) and E(Z) (22), in the functionally related *Drosophila* protein SU(VAR) 3–9 (23), in the ALL-1-related protein ALR (24), and in a series of other proteins from yeast (25), fungi, *C. elegans*, plants, and humans (24, 25). A TRX C-terminal segment containing amino acid residues 3375–3759, which span the SET motif and some upstream sequences, was inserted into the pGBT9 vector and used to screen a cDNA library prepared from 0 to 6 hr *Drosophila* embryos (26). Five positive clones were identified from 10^6 transformants, and one of these clones was selected for additional characterization. Sequence analysis indicated that the clone contained the complete ORF for the SNR1 protein linked in frame to the GAL4 transactivation domain. *snr1* was cloned previously (20) as the *Drosophila* homolog of the human ini1 (hsnf5) gene, which is related highly to yeast *snf5* (27, 28) and *snf5b* (21). INI1 and SNF5 are known components of the mammalian (21) and yeast (29, 30) SWI/SNF complexes, respectively. SNR1 coimmunoprecipitates from *Drosophila* extracts together with the product of the *trx*-G gene *brahm*a (BRM) and is present in a large ($>2 \times 10^6$ Da) complex, which presumably corresponds to *Drosophila* SWI/SNF. Most importantly, *snr1* genetically interacts with *trx* and *brahma*, suggesting cooperation in activating transcription of homeotic genes (20). To extend our findings to the corresponding mammalian genes, we applied the yeast two hybrid system and found that the ALL-1 C-terminal domain interacts with INI1. This interaction, as well as the one between TRX and SNR1, is specific. Neither SNR1 nor INI1 interact with the unrelated baits p53, LAMIN, ANKYRIN, DAP KINASE, a partial FAS protein, ICE, MORT-1, and TRAF-2. To precisely delineate the domains participating in these reactions, we generated a series of deletions encoding the TRX, ALL-1, SNR1, and INI1 polypeptides and tested them in the yeast interaction assay (Fig. 1). The shortest active segments of TRX and ALL-1 (amino acids 3540–3759 and 3745–3969, respectively) included the entire SET motif together with upstream stretches of ≈70-aa residues in each. Deletion of TRX SET amino acid residues 3652–3759 or 3704–3759, as well as of ALL-1 SET amino acid residues 3853–3969 or 3899–3969 resulted in the abolishment of the interactions with SNR1 and INI1, respectively (Fig. 1 *A* and *B*). The shortest SNR1 active region (amino acid residues 168–303) spanned two-thirds of the C-terminal sequence conserved with INI1 and SNF5. The shortest interacting INI1 fragment identified (residues 118–315) included

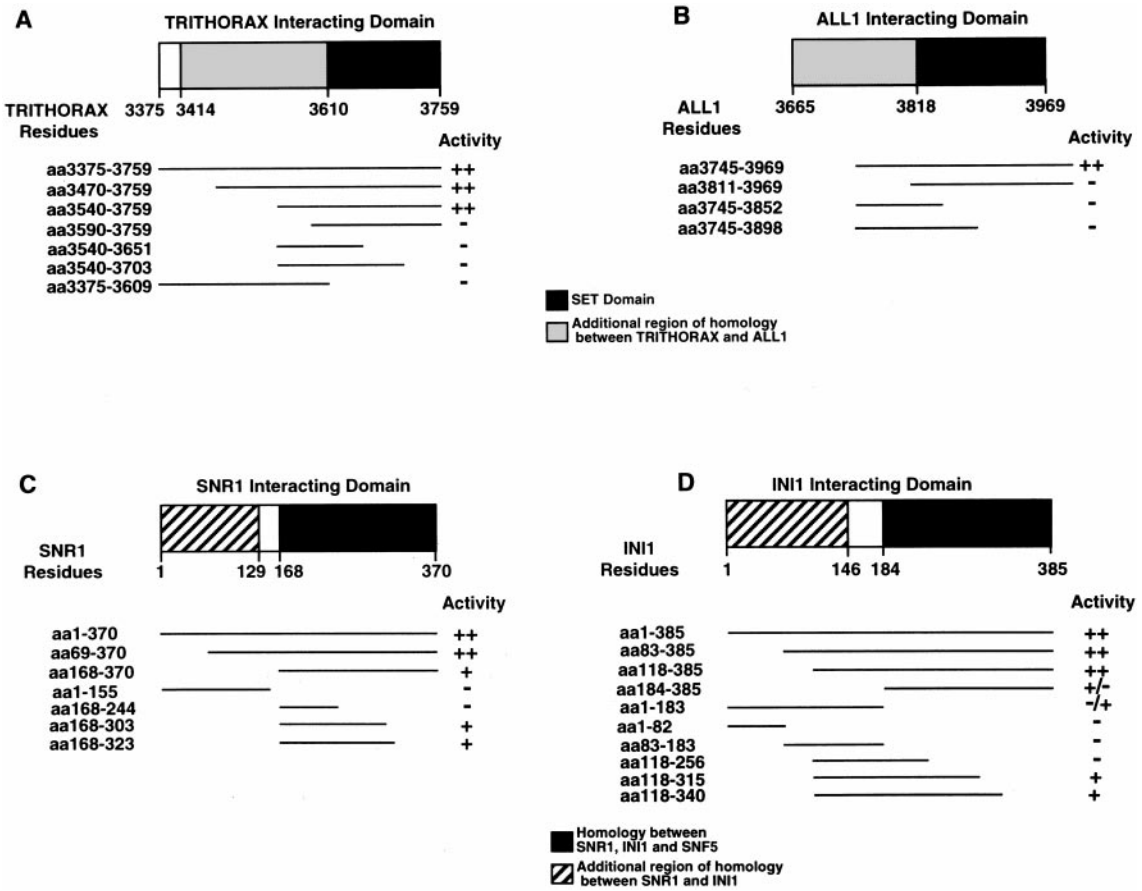

FIG. 1. Deletion mapping of interacting fragments. Assays of β-galactosidase were made in SFY526 yeast cells. Cotransforming fragments in *A*, *B*, *C*, and *D* were SNR1 (amino acid residues 1–370), INI1 (amino acid residues 1–385), TRX (amino acid residues 3540–3759), and ALL-1 (amino acid residues 3745–3969), respectively. ++, strong interaction; –/+, very weak interaction.

C-terminal sequences as well as additional upstream sequences. Subsequent work (see below), however, indicated that INI1 C-terminal domain (amino acid residues 184–385) is sufficient for coimmunoprecipitation with the ALL-1 C-terminal domain. We conclude that similar homologous sequences within the *Drosophila* and human proteins take part in both interactions.

To demonstrate *in vitro* binding, TRX and ALL-1 C-terminal fragments were linked to GST, expressed in bacteria, and bound to glutathione-Sepharose beads. SNR1 and INI1 proteins were synthesized and radiolabeled in a coupled transcription–translation system and examined for binding to the GST chimera polypeptides and to GST alone. Seven and fivefold more of the radiolabeled proteins were bound to GST-TRX and GST-ALL-1 fragments, respectively, compared with GST alone (Fig. 2*A*). Next, we attempted to confirm and extend these results by applying coimmunoprecipitation methodology. To this end, three SNR1 polypeptides and two TRX C-terminal polypeptides were tagged with the T7 and hemagglutinin (HA) epitopes, respectively. The SNR1 proteins were expressed transiently in cultured COS cells, either alone or in combination with one of the HA-tagged TRX constructs (Fig. 2*B*, lanes 5–13). Cell extracts were immunoprecipitated (IP) with anti-HA mAb, and SNR1 polypeptides were examined for coprecipitation by Western blotting and reaction with anti-T7 mAb. Expression and size of the SNR1 proteins was verified by IP with anti-T7 mAb followed by detection with the same reagent (Fig. 2*B*, lanes 1–4). Similarly, expression/IP of the TRX polypeptides was confirmed by IP and subsequent detection with anti-HA mAb (Fig. 2*B*, lanes 6′–12′). SNR1 was found to coimmunoprecipitate with two different TRX polypeptides (Fig. 2*B*, lanes 6 and 8), and two smaller SNR1 proteins also were coimmunoprecipitated with a TRX protein (Fig. 2*B*, lanes 10 and 12). The same methodology was applied to test for association between the C-terminal segment of ALL-1 and INI1. Indeed, three different polypeptides of INI1, tagged with the T7 epitope, were coimmunoprecipitated with the HA-tagged ALL-1 polypeptide (Fig. 2*C*, lanes 6, 8, and 10). We conclude that the TRX and ALL-1 C-terminal domains are associated physically with the SNR1 and INI1 proteins, respectively, which are overproduced in COS cells. The smallest active INI1 segment identified so far is similar to the region involved in direct or indirect association with a human BRM homolog, hbrm (28).

We now inquired whether full-length ALL-1 and INI1 are associated physically in cultured cells. The T7-tagged INI1 was transfected transiently into the human erythroid cell line K562. Nuclear extracts from transfected and untransfected cells were immunoprecipitated with Ab against the N terminus of ALL-1 (amino acid residues 79–290), with anti-T7 Ab, or with preimmune sera. The pellets were analyzed by Western blotting for the presence of T7-tagged INI1. INI1 was indeed found to coimmunoprecipitate with endogenous ALL-1 (Fig. 3*A*, lane 3). The presence of full-length ALL-1 in the pellet was verified by detection (Fig. 3*A*, lanes 10 and 11) with Ab raised against an ALL-1 polypeptide (anti-ALL-1C) spanning residues 2231–2389. To prove that INI1 precipitation was ALL-1-dependent, we expressed transiently the tagged INI1 protein in COS cells, in which full-length ALL-1 could not be detected previously, and subjected it to immunoprecipitation with anti-ALL-1N Ab. Neither INI1 (Fig. 3*A*, lane 8) nor ALL-1 (Fig. 3*A*, lane 12) were present in the precipitate. Thus, full-length endogenous ALL-1 is associated with INI1 *in vivo*.

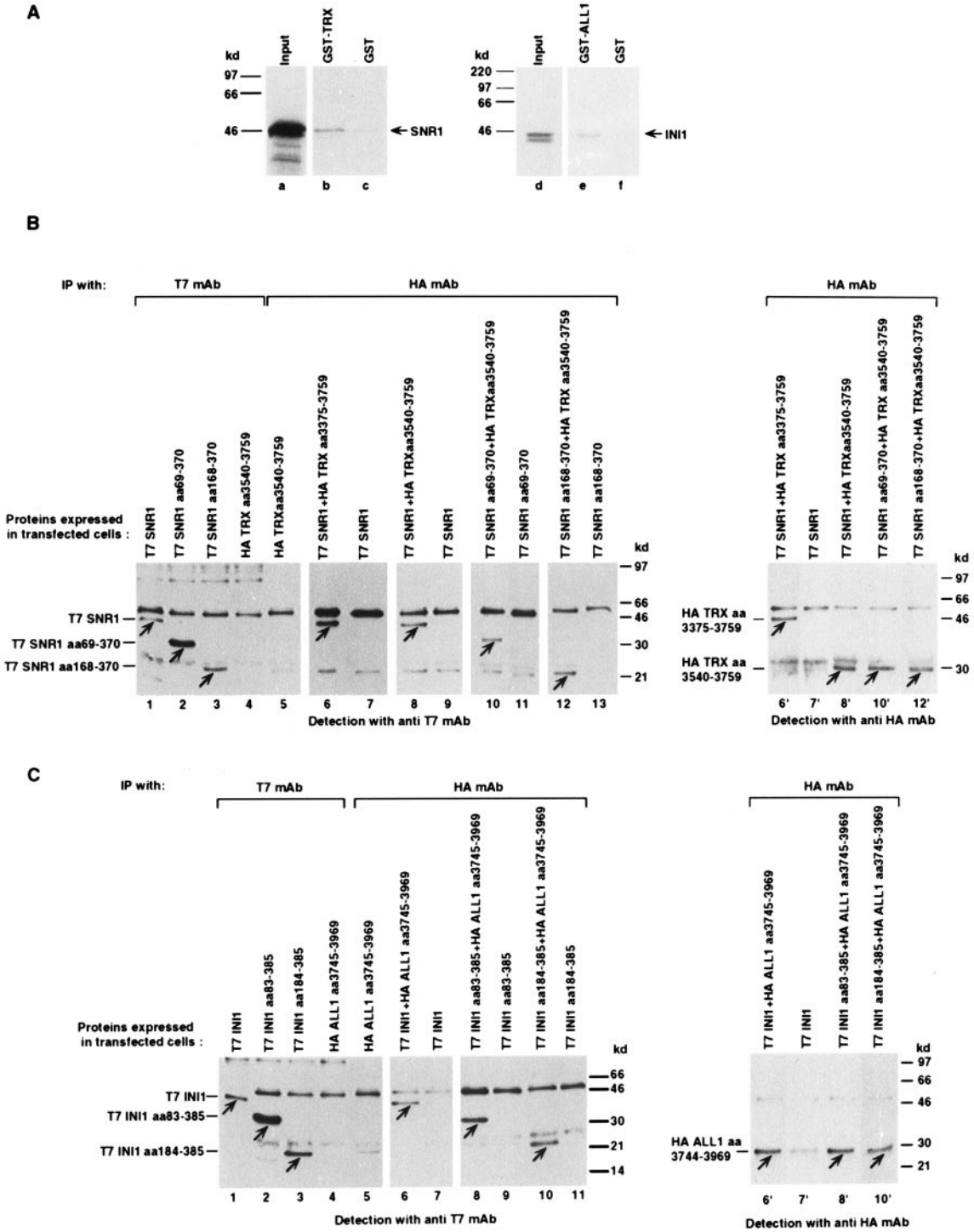

FIG. 2. *In vitro* (*A*) and *in vivo* (*B* and *C*) binding of TRX/ALL-1 polypeptides to SNR1/INI1. (*A*) TRX and ALL-1 segments spanned amino acid residues 3375–3759 and 3745–3969, respectively. Full length SNR1 and INI1 were synthesized and radiolabeled in a coupled transcription–translation system. Amount in input is 12% of the amount applied to beads. (*B* and *C*) Coimmunoprecipitation from COS cells of overproduced SNR together with TRX fragments and overproduced INI1 together with ALL-1 fragment, respectively. Equivalent amounts of extracts (including those analyzed in lanes 1–4 were immunoprecipitated, and proteins in the pellets were detected with anti-T7 or anti-HA mal.

To investigate whether full-length TRX and SNR proteins interact *in vivo*, we performed coimmunoprecipitation experiments with extracts prepared from adult flies. For this assay, expression of the HA-tagged version of the full-length SNR1 was directed by a heat shock (hs) promoter. Extracts from induced transgenic adult flies were incubated with either anti-TRX Ab or with other sera, and Ab–protein complexes were isolated by using protein A-coated beads.

Proteins in the pellet were analyzed by Western blotting with a mAb directed against the HA epitope. HA-SNR1 was expressed in the flies, as evidenced by its precipitation with anti-HA mAb. SNR1 protein was not detected in the pellet when preimmune serum was used for immunoprecipitation (Fig. 3*B*, *Left*), nor was SNR1 significantly immunoprecipitated with affinity-purified Ab against the PC or ENHANCER OF ZESTE [E(Z)] proteins (Fig. 3*B*, *Left*). In contrast, SNR1 was detected in the pellet when affinity-purified anti-TRX antibodies directed against the N-terminal region of the TRX molecule were used for immunoprecipitation (Fig. 3*B*, *Left*). Essentially, the same results were obtained when extracts were

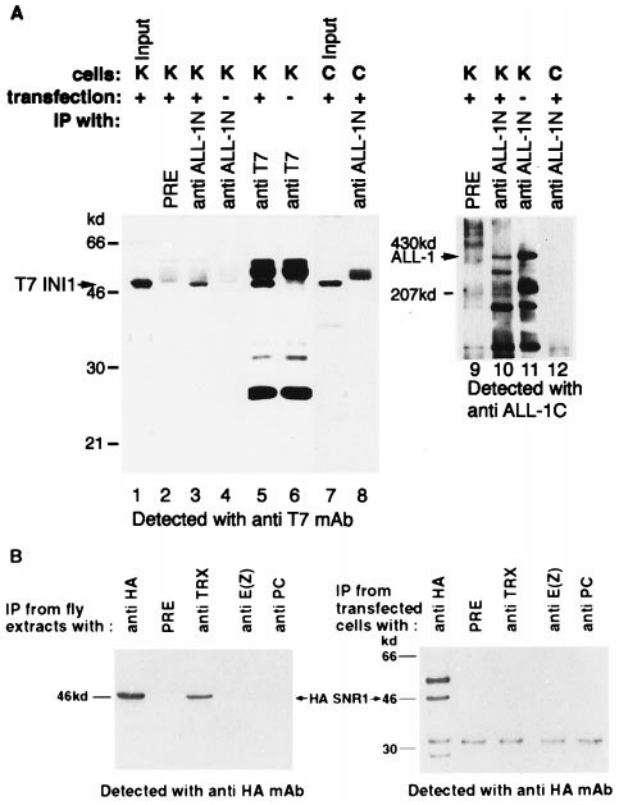

FIG. 3. Endogenous ALL-1 is coimmunoprecipitated from K562 cells together with overproduced INI1 (*A*). The product of the *snr1* transgene is coimmunoprecipitated from fly extracts with endogenous TRX (*B*, *Left*) and such SNR1 coimmunoprecipitation is TRX-dependent (*B*, *Right*). (*A*) T7-tagged INI1 was transiently expressed in human K562 cells (K) or in simian COS cells (C). Equivalent amounts of protein were subjected to IP. Input represents an aliquot of the nuclear extract analyzed directly by Western blotting (corresponds to 16% of the amount used for immunoprecipitation). (*B*, *Left*) Synthesis of HA-tagged SNR1 was induced by heat shock, and fly extracts were incubated with either anti-HA mAb, or with anti-TRX Ab and the corresponding preimmune sera (PRE), or with Ab against the E(Z) or PC proteins. Following precipitation, SNR1 was monitored by immunoblotting with anti-HA mAb. (*B*, *Right*) HA-SNR1 was expressed transiently in COS cells and subjected to immunoprecipitation and detection with the same Ab as in *B*, *Left*.

prepared from embryos following heat induction. To show that SNR1 immunoprecipitation was TRX-dependent, we transiently expressed HA-SNR1 in COS cells (which lack TRX) and tested for its coimmunoprecipitation with the five Ab (Fig. 3*B*, *Right*). Whereas SNR1 was expressed in the cells (Fig. 3*B*, *Right*, lane 1), it was not precipitated by anti-TRX or the other Ab. These results indicate that TRX and SNR1 are associated physically in both embryonic and adult fly extracts and are thus likely to interact *in vivo*.

Previously, we (12) and Chinwalla *et al.* (31) have localized the TRX protein to multiple sites on salivary gland polytene chromosomes of third instar larvae. Neither SNR1 nor BRM endogenous proteins have been reported to associate with polytene chromosomes, suggesting that their interactions with target genes might be transient or cannot be detected with the available methodology/reagents and that the BRM (SWI/SNF) complex is not associated tightly with chromatin. Nevertheless, we now have considered the possibility that, following overexpression, excess SNR1 protein, which is not associated with the BRM complex, might be available for binding to other chromosomal proteins such as TRX. To test this idea, we induced third instar larvae, transgenic for hsSNR1, and prepared salivary gland polytene chromosomes for immunostaining with antibodies. No SNR1 binding to polytene chromosomes was detected without heat treatment (not shown), but following heat induction and overexpression, SNR1 was found localized to several dozen discrete sites (Fig. 4, *Top*), suggesting specific binding to target genes. Previous immunostaining experiments with anti-TRX antibodies detected 16 strong bands and a number of weak bands (12), and this result is confirmed in Fig. 4, *Middle*. Double staining showed that TRX and hsSNR1 colocalized to around one-half of both strong and weak TRX binding sites (Fig. 4, *Bottom*), suggesting that these two proteins might indeed interact *in vivo*. Remarkably, in similar double-staining experiments using Ab against the *Pc*-G PC protein, which is localized to >100 bands on polytene chromosomes (10), we did not detect any overlap with HA-SNR1 binding sites (not shown), implying that SNR1 is not bound by PC. The partial overlap of TRX and SNR1 binding sites on polytene chromosomes suggests that the proteins are not required in concert for regulation of certain target genes and that both might have other interacting partners. TRX does

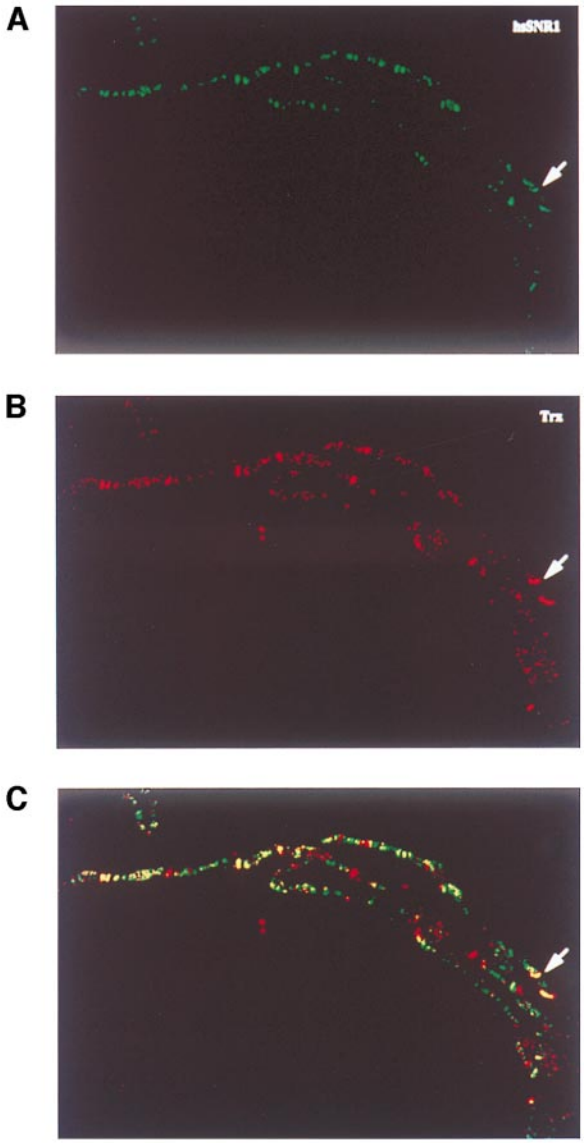

FIG. 4. Endogenous TRX and epitope-tagged SNR1 proteins colocalize on salivary gland polytene chromosomes of third instar larvae. Merging of green and red signals representing SNR1 and TRX respectively, identify the sites (yellow bands at the bottom) where the two proteins colocalize. Arrows indicate colocalization of both proteins at 98D1, the site of the endogenous *forkhead* gene.

not bind efficiently at the sites of the *Antennapedia* and bithorax loci on polytene chromosomes. This outcome is not surprising because these loci are not active in salivary glands. Similarly, we could not detect a strong SNR1 protein signal at the sites of these complexes. However, one of the strong TRX binding sites at 98D1 has been identified previously as the upstream regulatory region of the region-specific homeotic gene *fork head (fkh)*, and TRX has been shown to be required for *fkh* expression in embryonic and larval tissues (12). Significantly, TRX and SNR1 signals at 98D1 overlap (arrows in Fig. 4), suggesting that SNR1 as well as TRX might be bound to the *fkh* regulatory region. We note that direct evidence for the binding of TRX to multiple modules within the *bdx/pbx* regulatory region of the *Ubx* gene recently has been obtained (S.T., M. Fujioka, Y.S., T. Goto, and A.M., unpublished work) supporting the notion that TRX is associated physically with the maintenance element of an expressed target gene.

Taken together and in conjunction with the evidence that TRX is bound to chromatin at target sites, that TRX and SNR1 genetically interact and constitute positive regulators of HOM genes, and that SNR1/INI1 is a component of the SWI/SNF chromatin remodeling complex, our results suggest that TRX and ALL-1 recruit the SWI/SNF complex to target genes. We have not yet shown interaction between TRX/ALL-1 and SNR1/INI1 present within the SWI/SNF complex. However, such interaction might be difficult to demonstrate—inspection of the polypeptide patterns of mammalian SWI/SNF complexes purified from several cell lines (21) indicates an absence of the 430 K_d ALL-1 protein, implying that the latter is not an integral component of the SWI/SNF core. In addition, *Drosophila* BRM has never been shown to be attached to chromosomes. Thus, pairing between TRX/ALL-1 and the SWI/SNF-associated SNR1/INI1 might be transient, might involve a subset of the SWI/SNF complex molecules, or might occur in specific types of cells. Experiments are underway to investigate these possibilities.

The SET domain is the most striking element present within the segments of ALL-1 and TRX, which interact with INI1 and SNR1, respectively. SET is present in a series of other genes such as the *Drosophila trx-G/Pc-G* positive regulator *ash1*, negative/positive regulator *E(z)*, the yeast negative regulator *set1* (25), and the *Drosophila* supressor *Su(var)3–9*. The presence of the SET domain in positive and negative regulators in conjunction with the recent observation that hbrm, which usually is involved in gene activation as a component of the SWI/SNF complex, also acts as a corepressor of E2F1 (32), raises the question of whether interactions of SET domains with the INI1 and SNR1 proteins might entail both transcriptional activation and repression.

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