The RING Finger Protein SNURF Is a Bifunctional Protein Possessing DNA Binding Activity*

Received for publication, October 30, 2000, and in revised form, April 20, 2001
Published, JBC Papers in Press, April 23, 2001, DOI 10.1074/jbc.M009891200

Marika Hääki‡, Ulla Karvonen‡ Olli A. Jänne‡§, and Jorma J. Palvimo‡¶

From the ‡Biomedicum Helsinki, Institute of Biomedicine (Physiology), ‡Institute of Biotechnology, and the §Department of Clinical Chemistry, University of Helsinki, FIN-00014 Helsinki, Finland

The small nuclear C3HC4 finger protein (SNURF), RNF4, acts as transcriptional coactivator for both steroid-dependent and -independent promoters such as those driven by androgen response elements and GC boxes, respectively. However, SNURF does not possess intrinsic transcription activation function, and the precise molecular mechanism of its action is unknown. We have studied herein the interaction of SNURF with DNA in vitro. SNURF binds to linear double-stranded DNA with no apparent sequence specificity in a cooperative fashion that is highly dependent on the length of the DNA fragment used. SNURF interacts efficiently with both supercoiled circular and four-way junction DNA, and importantly, it also recognizes nucleosomes. An intact RING structure of SNURF is not mandatory for DNA binding, whereas mutations of specific positively charged residues in the N terminus (amino acids 8–11) abolish DNA binding. Interestingly, the ability of SNURF to interact with DNA is associated with its capability to enhance transcription from promoters containing GC box elements. Because SNURF can interact with both DNA and protein (transcription) factors, it may promote assembly of nucleoprotein structures.

RING1 (really interesting new gene) finger is a motif of conserved cysteines and histidines that coordinate two zinc atoms in a “cross-brace” system, a ligation scheme distinct from those of the classical zinc fingers (1, 2). The RING motifs can be classified into two subgroups according to the presence of a cysteine or histidine in the fifth position: C3HC4 (RING-HC) and C3HC3 (RING-H2) fingers. Otherwise their composition and length can vary substantially. The RING finger has been found in a variety of eukaryotic proteins of diverse evolutionary origin that are involved in various cellular processes such as oncogenesis, development, signal transduction, and apoptosis (1–3). RING fingers have been shown to mediate protein-protein interactions and formation of multi-protein complexes. The RING motif of promyelocytic leukemia gene product is important in the assembly of protein complexes linked to SUMO-1 (a small ubiquitin-like modifier protein) modifications (4). RING finger has also been suggested to act as a DNA-binding motif (5). The function of many RING-containing proteins can be mediated through DNA binding or chromatin association. RAG1 is involved in V(D)J recombination complex, and RAD-16 participates in DNA repair (6). RING finger-containing polycomb group proteins Psc, Su(z)2, Bmi-1, and RING1 are involved in the maintenance of the transcriptionally repressed state of genes by regulating chromatin structure (7–9), and Mel-18 is shown to act as a transcriptional repressor via binding to specific DNA sequence (10). Nuclear receptor mediator TIFα is tightly associated with euchromatin (11), whereas BRCA1 appears to be associated with the RNA polymerase II holoenzyme (12). However, the RING structures of these latter proteins have not been implicated in mediating their binding to chromatin or DNA.

Recent intriguing results have shown that many RING proteins are able to mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination in vitro (13, 14) and thus act as E3 ubiquitin ligases. However, as pointed out by Lorick et al. (13), only some of these proteins are likely to function primarily as E3s, and the RING finger-mediated ubiquitination probably provides many of these proteins with a self-regulatory function.

The small nuclear RING finger protein SNURF was initially identified as an androgen receptor (AR)-interacting protein by yeast two-hybrid screening. Rat SNURF is a highly hydrophilic protein composed of 194 amino acid residues of which about 30% are charged (15). The charge distribution of SNURF is asymmetrical; two negatively charged regions separate three basic amino acid clusters. The N terminus of SNURF encompasses a bipartite nuclear localization signal, and the C3HC4-type RING motif is localized in the C-terminal region. SNURF interacts with AR via its N-terminal region, whereas the RING finger plays a key role in the binding to promoter specificity protein 1 (Sp1) (16). In addition to AR and other steroid receptors, SNURF also enhances the activity of Sp1-regulated transcription. In contrast to many other coactivators, SNURF does not possess a conventional activation domain(s). To get better insight into the biological role of SNURF, we have studied the interaction of SNURF and its mutated forms with DNA. Our results indicate that SNURF possesses a general DNA binding activity that may explain some of its characteristics as a transcription-activating protein.

EXPERIMENTAL PROCEDURES

Materials—The BaculoGold transfection system and pAcG3X baculovirus transfer vector were purchased from PharMingen. Protease inhibitors phenylmethylsulfonyl fluoride (PMSF), leupeptin, pepstatin A, and aprotinin, as well as double-stranded calf thymus DNA cellulose...
were obtained from Sigma. Glutathione-Sepharose 4B, CM-Sepharose Fast Flow, single-stranded DNA agarose, Hybond-enhanced chemiluminescence (ECL) nitrocellulose membrane and ECL detection reagents were from Amersham Pharmacia Biotech. Horseradish peroxidase-conjugated anti-mouse IgG was from Zymed Laboratories Inc. Protein concentration was determined using BCA Protein Assay Kit purchased from Pierce. Synthetic DNA and labeled DNA oligonucleotides were purchased from Human Sp1 was from Promega. HMG-1 was purified from calf thymus (17) and histones were from rat thymus (18).

Production and Purification of SNURF—Recombinant viruses for wild-type and RING finger-mutated (C136S/C139S) rat SNURF GST fusion proteins were produced in Spodoptera frugiperda Sf9 cells by using Baculovirus transfection system (15). Sf9 cells were infected with recombinant virus at multiplicity of infection of 1 plaque-forming unit/cell, and cells were grown for three days. GST-SNURF proteins were extracted by sonication in a buffer containing 50 mM Tris-HCl (pH 7.5), 50 mM NaCl, 10% (v/v) glycerol, 0.5% (v/v) Triton X-100, 300 mM NaCl, 2 mM EDTA, 0.5 mM PMSF, 5 mM/ml leupeptin, 5 mM/ml pepstatin A, and 10 mM/ml aprotinin. The binding reactions were placed on ice for 30 min and then incubated with carrier DNA poly(dI-dC)2 (50 or 100 ng/reaction) on ice for 10 min. DNA-Cellulose Chromatography—Proteins (5 µg) were incubated with 20 µl of double-stranded (ds) calf thymus DNA cellulose (containing 300 mM NaCl, 10% (v/v) glycerol, 0.5% (v/v) binding buffer containing 20 mM Tris-HCl (pH 8.0), 20 mM NaCl, 0.5 mM DTT, and 10% (v/v) glycerol (19). After incubating at 4 °C for 4 h by rotation and washing four times with 1 ml of binding buffer for 4 h at 4 °C and subsequently washed four times with 1 ml of binding buffer containing 400 mM NaCl. Bound proteins were eluted by 0.2% (w/v) N-lauroylsarcosine (Sarkosyl) in a binding buffer containing 20 mM Tris-HCl (pH 7.8), 50 mM NaCl, 0.05% (v/v) Nonidet P-40, 50 µM ZnCl2, 0.1 mM DTT, 0.2 mM EDTA (pH 8.0), 0.5 mM PMSF, 5 µM/ml pepstatin A, and 10 µM/ml aprotinin and then with binding buffer without Sarkosyl. Sepharose beads containing 20 µg GST or GST-SNURF were incubated alone or with histones extracted from rat thymus and 0.5 µg of binding buffer containing 20 µg GST or GST-SNURF were incubated in 500 µl of binding buffer with 2 units/ml of streptomycin and penicillin. For transfection assays, 5 × 106 cells were seeded on 12-well plates 24 h prior to transfection using the FuGENE reagent (Roche Molecular Biochemicals). Four hours before the addition of DNA, the cells received fresh medium with 10% fetal bovine serum. After 8 h, the medium was changed to Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 25 units/ml of streptomycin and penicillin. For transfection assays, 5 × 106 cells were seeded on 12-well plates 24 h prior to transfection using the FuGENE reagent (Roche Molecular Biochemicals). Four hours before the addition of DNA, the cells received fresh medium with 10% fetal bovine serum. After 8 h, the medium was changed to Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Luciferase and β-galactosidase activities and the concentration of soluble cell proteins were assayed as previously described (15). For immunolocalization of SNURF mutants in COS-1 cells, cells grown on glass coverslips (1.6 × 105 cells on 6-well plates) were transfected with 750 ng of pcDNA-FLAG-SNURF or mutated SNURF forms in the same vector (25). Cells were processed, and immunofluorescence labeling and microscopy were performed as described in (25).

RESULTS

Expression and Purification of Recombinant SNURF—Recombinant GST-SNURF produced in Sf9 cells was adsorbed onto glutathione-Sepharose and cleaved with Factor Xa to remove the GST tail. In addition to the full-length SNURF migrating as ~34 kDa on SDS-PAGE (11), protease digestion produced smaller amounts of shorter polypeptides of ~26 kDa and ~16 kDa in size. N-terminal sequencing and mass spectrometric analysis indicated that the ~34 kDa product corresponded to the full-length SNURF. The two smaller polypeptides were identified as secondary cleavage products of SNURF by Factor Xa (26). Internal cleavages at Arg86 and at Arg181 produced an N-terminal SNURF fragment containing two stretches of basic amino acid residues and a C-terminal fragment encompassing the RING finger motif, respectively (Fig. 2). The proportion of the full-length SNURF in GST-cleaved preparation was ~60% of the total protein. In addition to SNURF fragments, the preparation did not contain other proteins in detectable amounts.
SNURF binds efficiently to various types of DNA—SNURF binding capacity toward supercoiled and linearized plasmid DNA was compared by using a gel retardation assay. GST-SNURF was incubated with supercoiled and linear pGEM-9Zf(−) DNA (2.9 kb) at various protein:DNA molar ratios, and protein-DNA interactions were monitored by agarose gel electrophoresis and ethidium bromide staining. SNURF bound to all the supercoiled DNA, and the mobility of SNURF-DNA complexes was progressively retarded with increasing SNURF concentrations (Fig. 3A), a phenomenon typical of general DNA-binding proteins (20). Similar mobility shift changes are not usually seen with sequence-specific DNA-binding proteins. SNURF retarded the mobility of both DNA types more efficiently than HMG-1. However, comparison of the interaction of SNURF between supercoiled and corresponding linear DNA did not reveal a clear preference for either DNA type (Fig. 3). GST alone did not interact with DNA.

To study further the DNA binding characteristics of SNURF, the interaction of SNURF with 4H DNA was examined and compared with that of HMG-1 (31, 32). EMSAs on parallel polyacrylamide gels were used to compare the binding of SNURF and HMG-1 to 4H DNA and linear DNA (a 232-bp restriction fragment). \(^{32}\)P-Labeled DNA fragments were incubated with increasing concentrations of SNURF or HMG-1. As shown in Fig. 4A, SNURF bound to 4H DNA and formed a weak DNA complex already at \(< 10 \text{ nM} \) protein concentration (lane 2). Most of the probe was up-shifted at \(40 \text{ nM} \) SNURF (lane 4), and an increase in the amount of protein up-shifted the complex further (lanes 5 and 6). Interestingly, HMG-1 bound to 4H DNA less efficiently than SNURF, and the complexes of HMG-1 were less stable during electrophoresis than those formed by SNURF. With linear DNA, progressive retardation of DNA was observed when the amount of SNURF was increased (Fig. 4B), indicating that, as in the case of 4H DNA, more than one SNURF molecule can interact with the same DNA molecule simultaneously. Comparable DNA binding pattern was obtained with GST-SNURF (results not shown), ruling out that the retardation pattern was due to SNURF fragments in protein preparations.

The Cooperativity of SNURF DNA Binding Is Influenced by the Length of the DNA Fragment—To study the influence of the length of target DNA sequence on the SNURF-DNA interaction, the ability of SNURF to bind to DNA fragments of varying lengths was compared by using \(^{32}\)P-labeled linear DNA probes.
in EMSA. Fixed amounts of labeled DNA of 33, 65, and 135 bp in length were incubated with increasing amounts of SNURF, and DNA-protein complexes were analyzed with EMSA. When the 33-bp DNA fragment was used, only \( \approx 40\% \) of the probe was up-shifted at 42 nM SNURF concentration, and the position of the complexes suggested presence of one or maximally two SNURF molecules bound to DNA (Fig. 5, lane 2). In contrast, the 65-bp and 135-bp DNA fragments were completely shifted at the same protein concentration (Fig. 5, lanes 6 and 10) and progressive retardation DNA was observed when 200 nM SNURF was used (Fig. 5, lanes 7 and 11), indicating that multiple SNURF molecules can interact with DNA fragments longer than \( \approx 60 \) bp. However, the SNURF-DNA complexes formed with the 135-bp DNA appeared to be more stable than those generated with the 65-bp fragment.

The N-terminal Basic Amino Acid Cluster of SNURF Is Critical for DNA Binding—To elucidate the regions of SNURF mandatory for the interaction with DNA, EMSAs were performed with mutated SNURF proteins expressed in *Epicurian coli* and purified as GST fusion proteins. GST-SNURF(C136S/C139S), in which two of the N-terminal cysteines of the RING finger (Fig. 2) were converted to serines, interacted with the \(^{32}\)P-labeled 232-bp DNA fragment as efficiently as wild-type GST-SNURF (Fig. 6), indicating that an intact zinc-coordinated RING structure is not mandatory for the interaction of SNURF with DNA. The deletion mutant lacking the second basic amino acid cluster (SNURFΔ66–98) bound to DNA as efficiently as full-length SNURF, whereas a C-terminal deletion mutant of SNURF (Δ178–194) lacking the third basic stretch formed slightly more labile DNA complexes (Fig. 6). In

![Figure 3](image3.png)

**FIG. 3.** Binding of SNURF to supercoiled and linear DNA as analyzed by electrophoretic mobility shift assay. Indicated molar protein:DNA ratios of GST-SNURF, HMG-1, or GST were incubated with negatively supercoiled pGEM-9Zf(-)A or with the corresponding linearized DNA (B) as described under “Experimental Procedures.” Protein-DNA complexes were resolved by electrophoresis on 0.7% agarose gels containing 1× Tris-phosphate-EDTA and visualized by ethidium bromide staining.

![Figure 4](image4.png)

**FIG. 4.** SNURF can interact with four-way junction DNA. A, increasing concentrations of SNURF (6–140 nM) or HMG-1 (20–550 nM) were preincubated with 50 ng of poly(dI-dC) for 10 min at 0 °C prior to the addition of \(^{32}\)P-labeled 4H DNA, and the incubation was continued for 30 min at 22 °C. B, \(^{32}\)P-labeled linear 232-bp DNA was used instead of 4H DNA under identical conditions. Protein-DNA complexes were separated on 4% nondenaturing polyacrylamide gels followed by autoradiography. F→, free DNA.

![Figure 5](image5.png)

**FIG. 5.** DNA binding of SNURF is dependent on the length of the target DNA fragment. Indicated concentrations of SNURF were first incubated with 100 ng of poly(dI-dC), and subsequently with \(^{32}\)P-labeled double-stranded DNA of 33 bp (lanes 1–3), 65 bp (lanes 5–7), or 135 bp (lanes 9–11) in length as described under “Experimental Procedures.” Protein-DNA complexes were resolved by electrophoresis on 4% polyacrylamide gels under nondenaturing conditions and visualized by autoradiography.
contrast, the deletion of amino acids 1–20, including most of the N-terminal basic amino acid cluster, blunted the ability of SNURF to interact with DNA.

To assess the role of this latter basic amino acid-containing stretch in DNA binding, arginines and lysines in the region were individually or together converted to alanines, and the corresponding GST fusion proteins were analyzed by EMSA. As shown in Fig. 6, mutation of neither Lys9, Arg10, nor Arg11 alone reduced significantly the DNA complex formation (lanes 10–12), whereas conversion of Arg8 to Ala resulted in clearly more labile DNA complexes. When Arg8, Lys9, Arg10, and Arg11 were all changed to alanines, the compound mutant (R8–11A/K9A) bound to DNA as poorly as the deletion mutant SNURFΔ1–20. Arginine 18, 23, and 25 as well as lysine 22 do not appear to play a critical role in DNA binding because a SNURF mutant having these amino acid residues mutated to alanines was still capable of binding to DNA. Taken together, these data indicate that amino acids 8–11 are the principal residues responsible for contacting DNA and that Arg8 plays a special role in the DNA binding of SNURF.

**SNURF Binds to Nucleosomes**—To examine whether SNURF can also interact with nucleosomes, EMSAs were performed with increasing amounts of GST-SNURF or SNURFΔ1–20 and a constant concentration of nucleosomes that were reconstituted with a 32P-labeled 164-bp DNA fragment. Although the mononucleosome fraction was purified by glycerol gradient centrifugation, some free DNA was present in the preparation, which is depicted as the band migrating faster than that of mononucleosomes (Fig. 7A, lane 1). This minor contamination of the mononucleosome fraction with free DNA was, in fact, beneficial as it permitted comparison of the binding of SNURF to naked DNA and to nucleosomes in the same assay. As illustrated in Fig. 7, GST-SNURF or GST immobilized onto glutathione-Sepharose was incubated with or without rat thymus histones for 4 h at 4 °C, and the matrices were washed extensively with buffer containing 0.4 M NaCl as described under “Experimental Procedures.” Bound histones were eluted with 0.2% Sarkosyl and resolved by electrophoresis on 15% SDS-PAGE. Lane 1, 10% of the input histones; lanes 2 and 4, histones eluted from GST-SNURF and GST matrices, respectively; and lanes 3 and 5, elutions from GST-SNURF and GST matrices not incubated with histones, respectively.

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**Fig. 6.** The positively charged amino acids in the N terminus of SNURF are required for DNA binding. A, GST-SNURF together with GST fusions of SNURF mutants C138S/C139S, Δ1–20, Δ66–98, Δ178–194, R8–11A/K9A, R8A, K9A, R10A, and R11A (100 nM each) produced in *E. coli* were compared for their ability to bind to 32P-labeled linear 232-bp DNA under conditions described in Fig. 4. B, SDS-PAGE analysis of the purified GST fusion proteins used in the EMSA experiment. Proteins (1 μg) were detected by Coomassie Brilliant Blue staining. F → free DNA.

**Fig. 7.** SNURF binds to nucleosomes. A, 32P-labeled in vitro reconstituted mononucleosomes were incubated with increasing concentrations (nM) of GST-SNURF (lanes 2–8), or the SNURFΔ1–20 mutant (lanes 9–15). Samples were analyzed on a 4% nondenaturing polyacrylamide gel at 4 °C and visualized by autoradiography. Arrow and arrowhead depict the position of unbound nucleosomes and free DNA, respectively. B, GST-SNURF or GST immobilized onto glutathione-Sepharose was incubated with or without rat thymus histones for 4 h at 4 °C, and the matrices were washed extensively with buffer containing 0.4 M NaCl as described under “Experimental Procedures.” Bound histones were eluted with 0.2% Sarkosyl and resolved by electrophoresis on 15% SDS-PAGE. Lane 1, 10% of the input histones; lanes 2 and 4, histones eluted from GST-SNURF and GST matrices, respectively; and lanes 3 and 5, elutions from GST-SNURF and GST matrices not incubated with histones, respectively.

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2 M. Hakki, O. A. Jänne, and J. J. Palvimä, unpublished observations.
12-well plates were transfected using FuGENE reagent with 160 ng of Sp12-TATA-LUC, 60 ng of β-galactosidase expression vector (pCMVβ), and 300 ng of indicated SNURF expression plasmids in pcDNA3.1(+)–FLAG backbone. After a 40-h culture, the cells were harvested, and luciferase activities in the cell extracts were adjusted to the transfection efficiency using β-galactosidase as an internal control. The reporter gene activity in the presence of empty pcDNA3.1(+)–FLAG vector was set as 1. The mean ± S.E. values of at least six experiments is shown.

Inset, Immunoblot analysis of wild-type and mutant SNURF proteins in an experiment corresponding to data shown in panel A. SNURF proteins were immunoblotted from the same lysates (pooled triplicate dishes) from which reporter gene activities were measured with an anti-SNURF antibody (15) and using the ECL system. Localization of wild-type SNURF (B), SNURFΔ1–20 (C), SNURFR8–11A/K9A (D), and SNURFR8A (E) in COS-1 cells. Cells grown on glass coverslips on 6-well plates were transfected with 750 ng of pcDNA-FLAG-SNURF or the corresponding vector encoding mutant SNURF forms. After a 40-h culture, cells were fixed with paraformaldehyde (4%) and permeabilized in 0.1% Triton X-100. SNURF antigen was detected by using anti-FLAG M2 monoclonal antibody followed by fluorescein isothiocyanate-conjugated secondary anti-mouse antibody, and immunofluorescence was recorded by using Bio-Rad MRC-1024 confocal laser system connected to Zeiss Axiovert 135 microscope.

The Ability of SNURF to Stimulate Transcription Correlates with Its DNA Binding Activity—We have previously shown that ectopic expression of SNURF activates minimal promoters containing Sp1-binding sites in front of a TATA box (15, 16). SNURF and Sp1 are also able to cooperate on natural promoters containing GC box elements, such as the rat p75 neurotrophin receptor promoter corresponding to the same 232-bp fragment as that used as the target DNA sequence in the preceding EMSA experiments (15). To assess further the importance of the N-terminal basic amino acids of SNURF in transcriptional activation, COS-1 cells were transfected with Sp12-TATA-driven luciferase (LUC) reporter (15) along with wild-type or N-terminally mutated SNURF forms by immunocytochemical analysis in COS-1 cells. As shown in Fig. 8B, D and E, the subcellular localization of neither SNURFR8A nor SNURFR8–11A/K9A differed from that of the wild-type protein. Although the deletion of the first 20 amino acids of SNURF (the SNURFΔ1–20 mutant) resulted in more cytoplasmic staining than seen with the other SNURF forms, it did not prevent the truncated protein from entering the nuclei (Fig. 8C). In view of these data, the inability of the DNA binding-deficient SNURF forms to activate transcription from Sp1 element-containing promoters is not attributed to their altered subcellular localization.

To examine whether SNURF and Sp1 can bind at the same time to the 232-bp rat p75 neurotrophin receptor promoter fragment, the 32P-labeled DNA was incubated with purified Sp1 alone or together with increasing concentrations of GST-SNURF, and the DNA-protein complexes were separated by EMSA. In the absence of SNURF, Sp1 formed one major and one minor complex with the probe (Fig. 9, lane 1). When a concentration of SNURF that alone bound the probe only weakly (lane 5) was included with Sp1, a third supershifted DNA complex became visible (Fig. 9, lane 2). When higher concentrations of SNURF were used with Sp1, most of the probe was gradually retarded to the position of the latter supershifted DNA-protein complex (Fig. 9, lanes 3 and 4), and the phenomenon was dependent on an intact N terminus of SNURF (Fig. 9, lanes 8–10). In accordance with the restricted ability of SNURF to interact with short DNA fragments, SNURF did not promote Sp1-DNA interaction when the GC box was embedded in a short oligomer (2). In sum, these results demonstrate that SNURF and Sp1 may interact concomitantly with the same target DNA sequence, although there was no clear cooperative effect by SNURF on the DNA binding of Sp1 under these in vitro conditions. Promotion of Sp1-GC box interaction cannot, however, be ruled out as a possible explanation for the stimulatory action of SNURF on Sp1-dependent transcription in intact cells.

![Transcriptional Coregulator SNURF and DNA Binding](image)

The ability of SNURF to stimulate transcription correlates with its DNA binding activity. We have previously shown that ectopic expression of SNURF activates minimal promoters containing Sp1-binding sites in front of a TATA box (15, 16). SNURF and Sp1 are also able to cooperate on natural promoters containing GC box elements, such as the rat p75 neurotrophin receptor promoter corresponding to the same 232-bp fragment as that used as the target DNA sequence in the preceding EMSA experiments (15). To assess further the importance of the N-terminal basic amino acids of SNURF in transcriptional activation, COS-1 cells were transfected with Sp12-TATA-driven luciferase (LUC) reporter (15) along with wild-type or N-terminally mutated SNURF forms by immunocytochemical analysis in COS-1 cells. As shown in Fig. 8B, D and E, the subcellular localization of neither SNURFR8A nor SNURFR8–11A/K9A differed from that of the wild-type protein. Although the deletion of the first 20 amino acids of SNURF (the SNURFΔ1–20 mutant) resulted in more cytoplasmic staining than seen with the other SNURF forms, it did not prevent the truncated protein from entering the nuclei (Fig. 8C). In view of these data, the inability of the DNA binding-deficient SNURF forms to activate transcription from Sp1 element-containing promoters is not attributed to their altered subcellular localization.

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3 H. Poukka, O. A. Jänne, and J. J. Palvimo, unpublished observations.
**DISCUSSION**

The rat RING finger protein SNURF was originally isolated as an androgen receptor-interacting protein in a yeast two-hybrid screen (15). The corresponding protein in human and mouse has been termed RNF4 (33, 34). These sequences are highly conserved, exhibiting 96% identity between rat and mouse protein and 91% identity between rat and human. Interestingly, no obvious SNURF/RNF4 orthologs are found in Caenorhabditis elegans, or Saccharomyces cerevisiae. In addition to interacting with steroid receptors and Sp1 (15, 16), RNF4 has been recently shown to associate with Gscl (goosecoid-like) homeodomain transcription factor (34), the activator of stromelysin I gene transcription (SPBP) (35), and a novel member of the BTB/POZ family PATZ (36). Interaction between SNURF and Sp1 or SPBP is mediated through the RING finger (15, 16, 35), whereas AR and Gscl are interacting with a region N-terminal to the RING finger (15, 16, 34).

SNURF is able to act as a transcriptional coactivator for different transcription factors (15, 16, 35), and conversely, to enhance transcriptional repression elicited by PATZ (36). The actual molecular mechanism of transcriptional regulation by SNURF is not known as no clear-cut intrinsic transcription activation or repression function has been found for SNURF. One potential explanation for the effects of SNURF is that it associates with DNA. Our results indicate that SNURF indeed possesses a general DNA binding ability without an apparent nucleotide sequence specificity. Interaction of SNURF with dsDNA was found to be more resistant to ionic strength than that of another non-sequence-specific DNA-binding protein, HMG-1. SNURF was also able to bind to single-stranded DNA. A similar behavior has been previously reported for HMG-1 and HMG-2 as well as for HMG-14 and HMG-17 (37). These HMG proteins are thought to function as architectural proteins that modify the structure of chromatin to generate conformations that facilitate or enhance various other DNA-dependent activities (27). Some non-sequence-specific DNA-binding proteins, such as Ku autoantigen, recognize preferentially DNA termini (38), which was a property, however, not inherent to SNURF. With linear DNA molecules, the cooperative DNA binding mode of SNURF correlated with the length of the DNA fragment in a fashion that resembles the binding characteristics of heterochromatin protein 1 (39). Heterochromatin protein 1, HMG-14, and -17, SNURF also is capable of binding to nucleosomes (27, 37, 39). In this regard, it is interesting that SNURF can recognize four-way junction DNA and that it shows binding preference for core histones H3 and H4. Binding to these DNA structures is a common property of many architectural proteins, and a wide variety of structurally and functionally unrelated DNA-binding proteins have been shown to bind preferentially to four-way junction DNA (40). Intriguingly, HMGI(Y), that favors binding to the four-way junctions, has been reported recently to associate with SNURF/RNF4 (36).

Besides the RING structure and the two nuclear localization signals (a bipartite-type and an SV40-type), no other protein motifs have been identified in SNURF sequence (Fig. 2). Our results show that the intact RING finger motif is not essential for the ability of SNURF to bind to DNA, further supporting the role of this structure as a protein-binding motif (16). Mutational analyses of the basic amino acid cluster (RKKRR11), present in the N terminus of SNURF and overlapping with the bipartite nuclear localization signal, revealed that this sequence is primarily responsible for the interaction with DNA and binding to nucleosomes. Of the individual residues, Arg8 plays a key role in DNA binding. These results suggest that the SNURF-DNA interaction is mainly electrostatic in nature. Interestingly, this type of Arg-rich cluster in the Drosophila homeodomain protein Bicoid is necessary for the recognition of both DNA and RNA targets (41), and similar motifs are found in a class of RNA-binding proteins (42). It is, therefore, an intriguing possibility that, like the Bicoid, SNURF is capable of recognizing RNA targets as well.

In addition to abolishing the DNA binding of SNURF, mutagenesis of the N-terminal basic amino acids also blunts the ability of the protein to stimulate transcription even though this particular SNURF domain is not centrally involved in heterologous protein interactions (15, 16). The relative DNA binding activity of various SNURF mutants correlated well with their ability to enhance transcription, strongly suggesting that interaction with DNA is essential for SNURF coactivator function. It is also worth pointing out that the mutations in the N terminus of SNURF did not alter nuclear localization of the proteins. Because also the RING finger-disrupted SNURF(C136S/C139S) interacts with DNA but is incapable of activating Sp1-mediated transcription (16), the binding to DNA cannot be the sole mechanism governing the ability of SNURF to activate transcription.

Taken together with our previous results, these data imply that SNURF enhances Sp1 activity through a combinatorial effect, involving protein-protein interaction and DNA binding (15, 16). SNURF may function through a similar mechanism also with other transcription factors such as Gscl (35). These results are reminiscent of the ability of HMG-1 to stimulate many sequence-specific transcription factors including steroid receptors (27, 43). Interestingly, a non-sequence-specific DNA binding activity has been recently shown for many coregulatory proteins implicated in steroid receptor function such as Hap46, TLS/FUS, and C1D/SUN-CoR (44–48). However, we have been unable to detect an unequivocal augmentation of AR DNA binding domain-DNA interaction by SNURF in vitro. This may reflect the requirement of AR regions outside the DNA binding domain for an efficient interaction with SNURF (15). With regard to AR, our additional experiments have shown that SNURF influences nuclear compartmentalization of the receptor (25). The role of DNA binding activity of SNURF in this process remains to be elucidated.

In conclusion, our data suggest that SNURF is a bifunctional protein that can interact both with transcription factors and...
with DNA/nucleosomes, thereby promoting the assembly of nucleoprotein structures involved in transcriptional control. A similar bifunctional activity may be a feature common to many transcriptional coregulatory proteins.

Acknowledgments—We thank Marc Baumann for peptide sequencing and mass spectrometric analysis, Hetti Poukka for plasmids, Orjan Wrango for help with nucleosomes, and Kati Saastamoinen and Seija Maki for technical assistance.

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