SUG1, a Putative Transcriptional Mediator and Subunit of the PA700 Proteasome Regulatory Complex, Is a DNA Helicase*

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Robert A. Fraser‡§, Mireille Rossignol‡®, David J. Heard†, Jean-Marc Egly, and Pierre Chambon**

From the Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), CNRS/INSERM/ULP, Collège de France, BP163 F-67404 Illkirch Cedex; C. U. de Strasbourg, France

Yeast SUG1 was originally characterized as a transcriptional mediator for the GAL4 transactivator. A similar role in vertebrates was suggested by the ligand-enhanced interaction between mammalian homologues of yeast SUG1 and the ligand-dependent activating domain (AF-2) of nuclear receptors. SUG1 was also shown to be a component of the PA700 regulatory complex of the 26 S proteasome and a member of a large family of putative ATPases. However, no catalytic function has yet been attributed to SUG1. We show here that SUG1 is a 3′-5′ DNA helicase whose activity is dependent on an intact ATP binding domain. The sedimentation heterogeneity of mammalian SUG1 suggests that it may be associated with distinct protein complexes and therefore play multiple roles.

Studies in transcription interference/squelching indicate that enhancement of transcription by nuclear receptors may require protein mediators (co-activators) linking ligand-activated nuclear receptors to the transcription machinery at promoters of target genes (1–3). Several putative transcription intermediary factors interacting in a ligand-dependent manner with the region containing the ligand-dependent transcription activation function-2 (AF-2) of nuclear receptors have been described recently (for review, see Ref. 4). Ligand-enhanced interaction between the AF-2 domain of nuclear receptors and either mouse (m)SUG1 (5) or its human homologue TRIP1 (6,7) has suggested that they may act as mediators in transcription activated by nuclear receptors. Moreover, yeast (y)SUG1, has been shown to suppress the effect of a mutation in the transcriptional activator GAL4 (8, 9). Functional similarity between yeast and mammalian SUG1 is supported by the ability of mSUG1 to rescue a conditional SUG1 mutation in yeast complementation experiments (5). Yeast SUG1 was also found in purified RNA polymerase II (pol II)1 holoenzyme complexes responsive in vitro to the transcriptional activators GAL-VP16 and GCN4 (10). However, this association is controversial as SUG1 does not appear to be present in the yeast holoenzyme preparation of Rubin et al. (11).

The sequence similarity between mSUG1, ySUG1, and TRIP1 is the highest in a region containing the consensus ATP binding site motif. This motif corresponds to an AAA module recently described for a large family of putative ATPases involved in a variety of cellular processes (for review, see Ref. 12). For instance, MSS1 and TBP1, which were originally identified as transcription factors, were later shown to be members of the PA700 proteasome regulatory complex of the 26 S proteasome (13–17). Similarly, Kominami et al. (18) and then Rubin et al. (11) found SUG1 in the yeast 26 S proteasome. This agrees with genetic evidence showing that yeast cells harboring a mutant allele of ySUG1 accumulate ubiquitinylated proteins normally degraded by 26 S proteasomes (19). A role for SUG1 in the regulation of the activity of the 26 S proteasome in vivo is further supported by the identical amino acid sequence of mSUG1 with the p45 subunit of the PA700 proteasome regulatory complex purified from human and bovine tissues, respectively (5, 17, 20).2 Therefore, SUG1 may be involved in more than one cellular function.

Despite the presence of a putative ATPase domain, no catalytic function has yet been attributed to SUG1. We show here that, in accordance with sequence similarities found with the DEHxD (where x = any amino acid) box subfamily of helicases (21), recombinant mouse SUG1 exhibits intrinsic 3′-5′ DNA helicase activity that is abolished by a mutation in its putative ATP binding domain. That SUG1 helicase may have multiple roles is supported by the observation that nuclear hSUG1 sediments not only with the PA700 complex, but also in regions corresponding to higher and lower molecular weights.

**EXPERIMENTAL PROCEDURES**

SUG1 Sequence Homology with Helicases—A computer search (BLAST (22)) was conducted with the full-length amino acid sequences of mSUG1 and two characterized helicases, XPB and XPD, to identify proteins with structural homology. Sequence comparison of mSUG1 with consensus helicase domains and known helicase XPB was done manually.

Purification of Recombinant His-SUG1—Plated S9 cells (400 × 10⁶) infected at a multiplicity of infection of 2 plaque-forming units/cell with baculovirus encoding a His-tagged SUG1 cDNA in the pAc5G HisNTB vector3 (PharMingen) were collected in growth medium, pelleted (3000

1 The abbreviations used are: pol II, polymerase II; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; Ni-NTA, nickel nitritotriacette; PAGE, polyacrylamide gel electrophoresis; mAb, monoclonal antibody; wt, wild type; IP, immunoprecipitation.
2 R. A. Fraser, D. J. Heard, and P. Chambon, unpublished results.
3 G. Weeda, M. Rossignol, R. A. Fraser, G. S. Winkler, W. Vermeulen, L. J. van’t Veer, L. Ma, J. H. J. Hoeijmakers, and J.-M. Egly, submitted for publication.
Extensively with wash buffer (binding buffer with 60 mM imidazole) and equilibrated nickel chelate (Ni-NTA) column. The column was washed with 500 mM imidazole elution fraction 2 (100 μl) and was expressed in and purified from h/SUG1 (see Fig. 2). SUG1 mAb (2SU1B8; Ref. 5) as follows: the fraction was precleared with 1 M KCl; the wash was dialyzed and tested (5 ml) for helicase activity (28). The Ni-NTA 500 mM imidazole elution fraction 2 (100 μl) was immunodepleted of His-SUG1 with an N-terminal-specific anti-His-mAb/protein G-Sepharose was washed with load buffer containing 500 μl of protein G-Sepharose in batch at 4°C for 1 h; the supernatant was collected and analyzed for SUG1 immunoreactivity; His-SUG1 bound to the mAb/resin was concentrated TFIIH (25) and equal volumes (5 ml) of the His-SUG1 (5 ml) for helicase activity and for SUG1 immunoreactivity; the His-SUG1 bound to the mAb/resin was washed with load buffer containing 500 μl of protein G-Sepharose in batch at 4°C for 1 h; the supernatant was collected and analyzed for SUG1 immunoreactivity; the His-SUG1 bound to the mAb/resin was then incubated with mAb bound to protein G-Sepharose at 4°C for 2 h; the supernatant was collected and analyzed for helicase activity (5 μl) and SUG1 immunoreactivity; the His-SUG1 bound to the mAb/protein G-Sepharose was washed with load buffer containing 500 μl of each 140 μl fraction were analyzed by Western blot and silver nitrate staining. SDS-PAGE—Equal volumes (20 μl for silver-stained and 5 μl for Western blot analysis) of the 500 μl of His-SUG1 as described above for wild type (wt) His-SUG1.

**RESULTS**

**Heparin Fraction 12** (5 ml) were separated centrifuged through a 20–40% glycerol gradient as described (23). 20 μl of each 140 μl fraction were analyzed by Western blot for the presence of hSUG1, the large subunit of RNA pol II (mAb 7G5), TFIIH subunit p62 (mAb 3C9 (27)) and PA700 subunits S7 (MSS1; mAb 2SCO) and S4 (mAb 4SCO). The glycerol concentration in each fraction was determined to ensure that the gradients were linear (data not shown).

**Antibody Production—Synthetic peptides** MPDYLGADQRTKEDKDDKPC and PGGKGDDEDKKSYYYEPVPC corresponding to hyperantigenic regions in the amino acid sequences of S7 and S4, respectively, were cross-linked to ovalbumin and injected into mice for the production of mAb 2SCO and 4SCO, respectively, as described previously (24).

**Glycerol Gradients—High molecular weight marker proteins (Pharmacia Biotech Inc.) and 150–200 μl (1.5–2 μg of protein) of a HeLa cell nuclear extract (28) were separately centrifuged through a 20–40% glycerol gradient as described (23). 20 μl of each 140 μl fraction were analyzed by Western blot for the presence of hSUG1, the large subunit of RNA pol II (mAb 7G5), TFIIH subunit p62 (mAb 3C9 (27)) and PA700 subunits S7 (MSS1; mAb 2SCO) and S4 (mAb 4SCO). The glycerol concentration in each fraction was determined to ensure that the gradients were linear (data not shown).

**Computer searches were performed with the full-length amino acid sequence of mSUG1 to identify proteins exhibiting sequence similarity.** Apart from the sequence matches to members of the AAA ATPase proteins, all data bases screened (PIR, Prodom, TrEMBL) revealed a significant similarity between SUG1 and two regions within the *Mycoplasma genitalium* homologue of the Holiday junction helicase RuvB (29). These regions of similarity (data not shown) overlapped with domains I and Ia (47% identity and 58% similarity over 34 residues) and II (31% identity and 75% similarity over 16 residues) of the seven conserved domains found in a DNA helicase family of proteins containing the DEH/D box (21). Some of these DEH/D box-containing proteins have been shown to have helicase activity (e.g. the helicase subunits of the repair/transcription factor TFIIH, XPD, and XPC (26, 30, 31)), and sequence analysis has revealed seven consecutive conserved domains; I, Ia (the putative ATP binding segment), II (the putative Mg2+ binding segment), III, IV, V, and VI (of unknown function) (21). No significant sequence similarity between SUG1, XPD, and XPC could be found using the BLAST program. Interestingly, apart from homologues in other organisms, a search with the BLAST program did not match XPD and XPC with sequences of other known helicases. However, some similarities were uncovered by manual alignment of the DEH/D box helicase consensus motifs (21) of SUG1 and XPD (30, 31) (Fig. 1B) or XPD data not shown; see Ref. 30). Note that in SUG1 the sequence similar to the consensus motif of domain VI is located between domains IV and V (Fig. 1A).
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**Fig. 2.** A recombinant His-SUG1 exhibited 3′-5′ DNA helicase activity. A, affinity-purified fractions consist primarily of a single protein species migrating with the size expected for His-SUG1, as indicated by the SDS-PAGE silver-stained gel (Silver Stained Gel). B, corresponding fractions were assayed for helicase (Helicase) activity and SUG1 immunoreactivity (Western blot; WB). SUG1 fractions exhibited a dose-related 3′-5′ DNA helicase activity as indicated by the displacement of the 24 nt-nucleotide fragment (compare Helicase and WB, lanes 1–4). Immunodepletion of His-SUG1 with anti-SUG1 mAb eliminated helicase activity (compare supernatant (s/n) in lane 5 with starting material, lane 2). Even after a 500 mM KCl wash (wash, lane 6) helicase activity was specifically retained on the protein G/anti-SUG mAb matrix (IP, lane 7). For comparison, TFIH was included for bi-directional helicase activity, buffer alone (−) to show background displacement of the substrates, and a 100 °C treatment (Δ) to show the amounts of 24- and 27-nucleotide-long substrates present in each reaction.

To investigate whether mSUG1 possesses helicase activity, recombinant His-SUG1 was expressed in S9 insect cells and purified to near homogeneity by DEAE-SpheroRed followed by nickel-chelate (Ni-NTA) affinity chromatography (Ni-NTA) and purified as above. Unlike wt His-SUG1, equimolar amounts of the mutant protein had no detectable helicase or ATPase activity (Fig. 3). The purification of the mutant and wt His-SUG1 was repeated several times, and in all cases the helicase and ATPase activities of each mutant preparation were negative in comparison with equimolar amounts of wt His-SUG1, thus eliminating the possibility that the helicase activity associated with wt His-SUG1 could be due to a co-purifying contaminant helicase. We conclude from these experiments that SUG1 is a 3′-5′ DNA helicase whose activity requires an intact ATP binding domain.

Monomeric SUG1 has a molecular mass of 48 kDa and is known to dimerize in vitro (5). To determine the relative size of endogenous SUG1 in the cell nucleus, human HeLa cell nuclear extracts were centrifuged through a 20–40% glycerol gradient. No further manipulations were performed on the crude extract to ensure that high molecular weight complexes remain intact (see Ref. 33). The sedimentation of HeLa cell SUG1 was compared with that of two other subunits of the PA700 complex (S7 (MSS1, molecular mass = 49 kDa) and S4 (molecular mass = 56 kDa) (14, 34)) and two components of the RNA pol II holoenzyme (the largest subunit of RNA pol II (molecular mass = 220 kDa) and the p62 subunit (molecular mass = 62 kDa) of TFIIH (27)) as well as markers of known molecular mass (Fig. 4). Most of the hSUG1 protein co-sedimented with the two other subunits, S7 and S4, of the PA700 complex (≈700 kDa) (Fig. 4, fractions 11–15). However, unlike the PA700 subunits S7 and S4, a portion of hSUG1 sedimented in fractions corresponding to much higher molecular masses that also contained a portion of the 220-kDa subunit of RNA pol II and of the p62 subunit of TFIIH (Fig. 4, fractions 22–26). Approximately 15–20% of nuclear SUG1 was also present in the low molecular mass range fractions (Fig. 4, fractions 2–7), in which very little S7 or S4 could be detected, thus indicating the possible existence of nuclear SUG1 complexes smaller than 700 kDa. Similar results were obtained with three different nuclear extracts (data not shown).

**DISCUSSION**

SUG1 is characterized here as a 3′-5′ DNA helicase based on the following biochemical evidence: (i) the helicase activity cofractionates with His-SUG1 over DEAE and affinity chromatographic steps; (ii) the level of helicase activity correlates with the amount of stained and immunoreactive His-SUG1 present in the chromatographic fractions; (iii) monoclonal an-
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FIG. 4. Sedimentation of HeLa nuclear extract through a 20–40% glycerol gradient indicates that endogenous hSUG1 is associated with multiple complexes within the cell nucleus. Upper panel, the relative sedimentation of the largest subunit of RNA pol II (RNA pol II), p62 subunit of TFIH (p62), the S4 and S7 subunits of the PA700 complex, and SUG1 was determined by repeated immunostaining of two Western blots (fractions 2–15 and 16–29, respectively). Positions of markers of known molecular mass are indicated at the top.

Like other proteins, immunoreactive SUG1 was found in all tested fractions with relative peak concentrations in the molecular mass ranges of 67–140 kDa (fractions 2–7) and 669 kDa (fractions 11–16). SUG1 was also present in very high molecular mass ranges (fractions 22–26). Lower panel, fractions 10–20 were re-run to clearly show that SUG1 co-sedimented with the PA700 complex, but was also present in fractions of higher and lower molecular mass.

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