The XPB subunit of repair/transcription factor TFIIH directly interacts with SUG1, a subunit of the 26S proteasome and putative transcription factor

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ABSTRACT

Mutations in the basal transcription initiation/DNA repair factor TFIIH are responsible for three human disorders: xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD). The non-repair features of CS and TTD are thought to be due to a partial inactivation of the transcription function of the complex. To search for proteins whose interaction with TFIIH subunits is disturbed by mutations in patients we used the yeast two-hybrid system and report the isolation of a novel XPB interacting protein, SUG1. The interaction was validated in vivo and in vitro in the following manner. (i) SUG1 interacts with XPB but not with the other core TFIIH subunits in the two-hybrid assay. (ii) Physical interaction is observed in a baculovirus co-expression system. (iii) In fibroblasts under non-replication conditions a portion of SUG1 is bound to the TFIIH holocomplex as deduced from non-overexpression conditions. (iv) In fibroblasts under non-replication conditions a portion of SUG1 is bound to the TFIIH holocomplex as deduced from non-overexpression conditions. (v) Purification of TFIIH to homogeneity demonstrated that it is a multisubunit protein complex that contains a minimum of nine proteins. (vi) TFIIH also has kinase activity specific for the large subunit of RNA polymerase II (1). Biochemical evidence in both yeast and mammalian cells has shown TFIIH to be part of the RNA polymerase II holocomplex. Included in this megadalton complex with all basal transcription factors except TFIID are the suppressor of RNA polymerase B (SRBs) proteins, transcriptional activators and mediators and the chromatin remodelling SWI/SNF factors (7–13).

The p89 and p80 subunits of TFIIH were found to be identical to the XPB and XPD helicases involved in nucleotide excision repair (NER) (3,4,14–17). The NER pathway removes a variety of structurally unrelated DNA lesions in a multi-step pathway (18). The consequences of inborn errors in NER are highlighted by the prototype repair syndrome, xeroderma pigmentosum (XP), an autosomal recessive condition displaying sun (UV) sensitivity, pigment abnormalities and predisposition to skin cancer (19). Two other distinct excision repair disorders have been recognized: Cockayne syndrome (CS) a neurodevelopmental, photo-sensitive condition and trichothiodystrophy (TTD) which resembles CS but shows, in addition, brittle hair and nails (20,21). The NER syndromes are genetically heterogeneous and comprise at least 10 complementation groups: seven in XP (XP-A to XP-G), five in CS, part of which overlap with XP complementation groups (CS-A, CS-B, XP-B, XP-D and XP-G) and three in TTD (TTD-A, and two XP groups; XP-B and XP-D) (18 and references therein). Strikingly, the proteins involved in XP-B, XP-D and TTD-A, i.e., all complementation groups with TTD features and most of the combined XP/CS groups, appear to be part of TFIIH (22). The dual function of TFIIH in repair and basal

INTRODUCTION

Transcription of protein encoding genes in higher eukaryotes by RNA polymerase II (Pol II) is a multi-step process involving a preinitiation complex containing several basal transcription factors, including TFIIH (for review see 1). An essential step is the conversion of a closed to an open initiation complex by local melting of the transcription start site probably due to the DNA-unwinding activity of the XPB and XPD subunits of TFIIH (2–5). Purification of TFIIH to homogeneity demonstrated that it is a multisubunit protein complex that contains a minimum of nine proteins (6). TFIIH also has kinase activity specific for the large subunit of RNA polymerase II (1). Biochemical evidence in both yeast and mammalian cells has shown TFIIH to be part of the RNA polymerase II holocomplex. Included in this megadalton complex with all basal transcription factors except TFIID are the suppressor of RNA polymerase B (SRBs) proteins, transcriptional activators and mediators and the chromatin remodelling SWI/SNF factors (7–13).
transcription and the specific link between mutations in this complex and the heterogeneous clinical features of CS and TTD, prompted the idea that at least part of the CS and TTD symptoms arise from (viable) defects in the transcription function of the complex. Thus, different mutations in the XBP and XPD helicases differentially affect the transcription and repair function of the complex giving rise to either XP, or XP/CS or TTD manifestations (22–24). In the case of XP-B patient XP11BE molecular analysis revealed a splice mutation in the XBP gene leading to a frameshift altering the last 41 amino acids of the encoded protein. Cells of this XP/Cs patient are almost completely deficient in NER (14). The enzymatic activity of TFIIH isolated from XP11BE lymphoblast cells is reduced in both the XBP-derived 3′→5′ helicase and in the in vitro basal transcription activity (25). Perhaps these effects are in part exerted by impaired protein–protein interactions. To identify proteins whose binding to XBP protein is diminished by the XP11BE mutation, we utilized the yeast two-hybrid system (26).

With XBP as a bait we identified SUG1 as a protein specifically interacting with TFIIH and whose affinity is diminished by the XP11BE mutation (27). SUG1 has previously been identified as a part of the 26S proteasome complex (27 and references therein) and interacting with TFIIH and whose affinity is diminished by the XP11BE mutation (27). SUG1 has previously been identified as a part of the 26S proteasome complex (27 and references therein) and part of the RNA polymerase II holocomplex (7).

MATERIALS AND METHODS

Cells and viruses

Spodoptera frugiperda clones 9 or 21 (S/9 or S/21) and the baculovirus transfer vector pVL1392 were purchased from PharMingen. Baculoviruses were propagated in insect cells at 28°C in Hinks insect medium supplemented with 10% fetal calf serum (FCS) and antibiotics. The human fibroblast cell line C5RO was grown in F10/DMEM medium containing 10% FCS and antibiotics. The human fibroblast cell line C5RO was grown in F10/DMEM medium containing 10% FCS and antibiotics. The human fibroblast cell line C5RO was grown in F10/DMEM medium containing 10% FCS and antibiotics. The human fibroblast cell line C5RO was grown in F10/DMEM medium containing 10% FCS and antibiotics. The human fibroblast cell line C5RO was grown in F10/DMEM medium containing 10% FCS and antibiotics.

Expression of the recombinant XBP and mSUG1 in insect cells

The BamHI fragment containing the entire coding region of XBP was inserted into pVL1392, yielding pVLXBP. The pVL-ERCC3 virus containing the entire XBP coding sequence with an eight-codon addition, including the codons for six histidine, at the 5′-terminus of the ORF was described in ref. 28. A Xhol–BamHI fragment comprising the coding region of mSUG1 was inserted into pAcSGHisA, yielding pAcSmSUG1. An 1.4 kb BamHI mSUG1 cDNA fragment tagged with the Hemagglutinin epitope (HA) of the influenza virus was ligated in the BamHI digested pVL1393 vector, yielding pVLMSUGHA.

Insect cells were co-transfected with a mixture of linearized AcNPV DNA (BaculoGold™) and transvector DNA as described according to the manufacturer’s protocol. Expression of recombinant His-tagged mSUG1, HA-tagged mSUG1, XPB, His-tagged XBP infected cells was determined by analyzing the cell extracts with immunoblotting.

For immunoprecipitations, monolayer insect cells (6-well plates) were (co)infected with recombinant virus at a multiplicity of infection (M.O.I.) of ~5 p.f.u. per cell. At 3 days post-infection cells were dislodged by pipetting and centrifuged at 2000 g for 3 min. Subsequently, they were washed twice with phosphate-buffered saline (PBS) and then resuspended in 1 ml E1A immunoprecipitation buffer (50 mM Tris–HCl pH 7.5, 50 mM NaCl, 5 mM EDTA, 0.1% NP-40), including 1 mM phenylmethylsulfonyl fluoride (PMSF), 50 μg/ml trypsin inhibitor and 1 μg leupeptin and pepstatin (referred as protease inhibitors).

For nickel-chelate affinity chromatography whole cell extract (WCE) was dialyzed against NTA binding buffer (20 mM Tris pH 7.9, 500 mM NaCl, 5 mM imidazole, 5 mM DTT and protease inhibitors) prior to application onto an equilibrated NTA column. The column was washed extensively with wash buffer (binding buffer with 60 mM imidazole) and then eluted into 0.5 ml column fractions with 100 and 500 mM imidazole containing binding buffer.

cDNA library screening and yeast transactivation assays

Construction of the 14.5 day-old CD-1 mouse embryo cDNA library in the yeast AAD fusion vector pPC96 is described elsewhere (29). The starting plasmid for construction of GAL4(DB) fusion vectors was pPC97. Generation of the GAL4 fusion vector that was used as bait to screen the cDNA library is constructed as follows. A 2.4 kb Smal–Nsi1 fragment containing the human XBP cDNA (14) lacking 30 amino acids at the N-terminus, was ligated in the Smal site of pPC97 (29), yielding pPC97E3. XBP cDNA plasmids containing the C-terminal truncations C21 and C42 have been described previously (30) and were subcloned into the GAL4 fusion vector pPC97 as described above. Similar constructs were generated fusing the other TFIIH core components with the GAL4 DNA-binding domain, including XBP–XP11BE (aa 31–742), XPB (aa 1–760), p62 (aa 24–548), p44 (aa 37–395), p52 (aa 1–462) and p34 (aa 22–303). Note that in all cases GAL4 fusion constructs have been tested for expression levels in the yeast strain BJ5459 (ura3-52, trpl, lys2-801, leu2Δ1, his3Δ200, pep4::HIS3 prb1Δ1,6R, can1, GAL) by immunoblotting using the antibodies against GAL4 DBD region (Ab 5C1, kindly provided by R. Bernards, Amsterdam) or the corresponding TFIIH subunit. The XBP bait in the pPC97 plasmid was introduced by LiAc transformation into the Saccharomyces cerevisiae Y190 reporter strain (MATa, leu2-3, 112, ura3-52, trpl-901, his3Δ200, ade2-101, gal4Δ40gal80ΔURA3Glu-LacZ, LYS GAL-HIS3, cyhΔ) (31). Approximately 1.5 × 10⁶ yeast transformants were selected on 40 9-cm plates of supplemented synthetic dextrose medium including 25 mM 1,2,4 triazol-3-ylamin 3-amino-1,2,4,-triazole lacking tryptophan, leucine and histidine. After 4–5 days, 39 transformants of which GAL4 activity had been reconstituted were isolated on 9-cm plates of synthetic dextrose medium including 25 mM 1,2,4 triazol-3-ylamin 3-amino-1,2,4-triazole lacking tryptophan, leucine and histidine. After 4–5 days, 39 transformants of which GAL4 activity had been reconstituted were isolated on 9-cm plates of supplemented synthetic dextrose medium including 25 mM 1,2,4 triazol-3-ylamin 3-amino-1,2,4-triazole lacking tryptophan, leucine and histidine. After 4–5 days, 39 transformants of which GAL4 activity had been reconstituted were isolated on 9-cm plates of supplemented synthetic dextrose medium including 25 mM 1,2,4 triazol-3-ylamin 3-amino-1,2,4-triazole lacking tryptophan, leucine and histidine.

Plasmids

The ~1.4 kb mSUG1 cDNA was subcloned into the eukaryotic expression vector pcDNA3 vector (Invitrogen), yielding pCMSUG1. Oligonucleotides p209 (5'-CCGGATCCAGATGG-
and incubated o/n at 4°C with 50 Ci/mM). cDNA at a concentration of 0.1 µg/µl was microinjected into one of the nuclei of polykaryons. Cells were assayed 24 h after microinjection.

**Immunological methods and antibodies**

Anti-XPB MAb (1B3) and anti-p62 MAb (3C9) are described elsewhere (3,38). MAb 2SU was raised against the N-terminal region of mSUG1 (aa 1–149) (39). MAb 3SU was raised against the C-terminal region of mSUG1 (40). MAb-CTmut was raised against influenza hemagglutinin peptide HA1 (75–110) (41). In some immunoprecipitation experiments antibodies were crosslinked to protein A—Sepharose.

Immunoprecipitation was performed at 4°C by addition of 40–100 µl of protein A/G—Sepharose (10%). The immunoprecipitated proteins were separated by 11% SDS–polyacrylamide gel electrophoresis (PAGE) and transferred to polyvinylidene difluoride membranes (Millipore) or nitrocellulose in 25 mM Tris–HCl–192 mM glycine buffer (pH 8.3) containing 20% methanol. Antibody bound antibodies were detected with a horseradish peroxidase-linked Goat anti-mouse IgG and an enhanced chemiluminescence detection system. For immunoprecipitation/imunoblot analysis, a horseradish peroxidase-linked anti-mouse IgG (Southern Biotechnology Associates Inc.) was used as secondary antibody.

**RESULTS**

**Isolation and characterization of mSUG1**

The wild-type human XPB (aa 31–742) was fused to the GAL4 DNA-binding domain (DBD) (aa 1–47) and used as bait in a two-hybrid screen for interacting proteins encoded by a library of cDNAs (mouse) and fused to the GAL4 activation domain (AD) (29). Yeast transformants (1.5 x 10⁶) were selected by plating on synthetic dextrose medium lacking tryptophan, leucine and histidine to select for those transformants that could express a Gal1-HIS3 gene which is regulated by GAL4 binding sites. His+ transformants were subsequently screened for the expression of a second reporter gene, GAL1-LacZ. Among the 39 His+ clones, one yeast clone was positive for LacZ expression. DNA sequence analysis revealed an open reading frame of 406 amino acids fused in frame with the GAL4 activation domain. The encoded protein appeared identical to the mouse protein, mSUG1 (39), a functional homolog of the yeast transcription factor/mediator SUG1 (42,43) and to p45 of the PA700 regulatory subunit of the functional homolog of the yeast transcription factor/mediator SUG1 (42,43) and to p45 of the PA700 regulatory subunit of the

**Microinjection**

Microneedle injection of wild-type human fibroblasts (CSRO) was performed as described earlier (37). RNA synthesis was determined by a 1 h pulse labeling with [3H]uridine (10 µCi/ml; s.a.: 50 Ci/mM). cDNA at a concentration of 0.1 µg/µl was microinjected into one of the nuclei of polykaryons. Cells were assayed 24 h after microinjection.
Figure 1. Sequence analysis of mSUG1 cDNA. mSUG1 is 100% identical to the human p45 protein component of the PA700 regulatory complex of the human 26 proteasome (44). Comparison of the predicted amino acid sequence of mSUG1, yeast SUG1 (39,42), MSS1, a positive modulator of HIV Tat-mediated transactivation (52) and the yeast homolog of MSS1, CIM5 (54), TBP1 (55) and the yeast homolog of TBP1 (YTA1) (56). Sequence identity is presented in black boxes, whereas similar residues (A, S, T, P and G; D, E, N and Q; R, K and H; I, L, V and M; F, Y and W) are given in grey boxes. Three amino acids differ between mSUG1 and the human published TRIP1 protein (45) (amino acid residues 266 S, 272 Q and 300 which are respectively D, T and I in SUG1 and p45, but S, Q and M in the Trip1 sequence). The putative functional domains are indicated. The fact that the aspartic acid (D), the threonine (T) at positions 266 and 272 are completely conserved in the AAA-family members from yeast to human and isoleucine (I) at position 300 is conserved between yeast and human SUG1 suggests that they are invariant and that the differences with the Trip1 protein might represent sequencing errors.
TFIIH components were fused to the GAL4 DNA-binding domain (aa 1–406) was fused to the GAL4 activation domain (AD) and other core subunits of TFIIH. For these experiments, mSUG1 (aa 62, 52, 44, 34 and a vector control were paired with SUG1 or SUG1 expressed alone (data not shown). Interestingly, the XP11BE mutant interacts with mSUG1, but generated a transcriptional signal 5–10 times lower than the wild-type XBP fusion protein (Fig. 2A). These data suggest that the C-terminal 41 amino acid nonsense part of the mutant XBP protein interferes with mSUG1 binding. To localize the region(s) required for the interaction with SUG1, truncation mutants of XBP, lacking the carboxy 21 and 42 amino acids residues (CA21 and CA42, respectively) were fused to the GAL4–DBD domain and assayed for functional interaction. All the fusion proteins were expressed at similar levels, as judged by immunoblot analysis (Fig. 2B) ruling out the possibility that reporter activity is reduced due to different expression levels of the DBD-fusion proteins. Both C-terminal truncated fusion proteins resulted in a decrease of interaction with mSUG1 compared to wild-type XBP. These data indicate that XBP C-terminus, though important, is not indispensable for functional interaction. It appears that the C-terminal frame-shifted nonsense sequence may interfere in a semi-dominant fashion with mSUG1 interaction, since the binding with the XP11BE mutant protein is even more affected.

mSUG1 interacts with XBP in vitro

To confirm the interaction detected in the two-hybrid system with a biochemical assay, XBP and His6-tagged mSUG1 were co-expressed in insect cells using the baculovirus system. Crude extracts infected with either XBP alone or in combination with his-tagged mSUG1 baculoviruses were loaded onto a nickel-chelate affinity column. After washing and elution with 100 and 500 mM imidazole, immunoblot analysis indicated that XBP is retained onto the affinity column only in the presence of His-tagged mSUG1, whereas XBP overexpressed alone mainly flowed through the column [Fig. 3A, compare the test column (XBP/mSUG1 lysate) with the control column (XBP lysate, lanes 4–11)]. It is worth noting that XBP-infected cell extracts do not contain any immunoreactive SUG1 (Fig. 3A, control column, lane 1). To further demonstrate the specificity of the interaction between XBP and SUG1, the 500 mM imidazole eluted fraction (2 (Fig. 3A, lane 10) was immunoprecipitated with a MAb-XPB antibody (1B3). Even after extensive washing with buffer containing 500 mM KCl, mSUG1 still remained associated with XBP (Fig. 3B, lane 3), whereas neither XBP nor SUG1 were retained on the control beads cross-linked to Mab GST (lane 5).

Since XBP interaction could also have been mediated through the His-tag we used an alternative approach (Fig. 4). Extract from insect cells co-infected with viruses expressing XBP and Hemagglutinin (HA)-tagged mSUG1 were immunoprecipitated with a MAb-XBP (1B3) or an anti MAb-SUG1 (2SU). Immunoprecipitates were analyzed on immunoblots with a MAb-XPB antibody (1B3). Even after extensive washing with buffer containing 500 mM KCl, mSUG1 still remained associated with XBP (Fig. 3B, lane 3), whereas neither XBP nor SUG1 were retained on the control beads cross-linked to Mab GST (lane 5).

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Figure 3. Purification of a SUG1–XPB complex from insect cells. Sf9 cells were infected with XPB or co-infected with XPB and mSUG1-His baculoviruses (pVLXPB and pAcmsSUG1, respectively) as described in Materials and Methods. Equal amounts of XPB/mSUG1-His and XPB lysates were loaded onto a nickel-chelated affinity test column and control column respectively and extensively washed with binding buffer containing 60 mM imidazole. The bound proteins were then sequentially eluted with 100 and 500 mM imidazole. (A) The load (lane 1), the flow through (FT) (lane 2), the wash (lane 3) and the eluted fractions (lanes 4–11) were analyzed by immunoblotting with MAbs against XPB (1B3) and SUG1 (2SU). (B) The 500 mM imidazole eluted fraction 2 [(A) lane 10 of the test column] was immunoprecipitated with either XPB MAb (1B3) or, as negative control, GST MAb crosslinked to protein A–Sepharose. The load (lane 1), the flow through (FT, lanes 2 and 4) as well as the proteins bound to control MAb (lane 5) and to XPB MAb (lane 3) were analyzed by immunoblotting and probed with MAbs against SUG1 (2SU) and XPB (1B3).

His-tag affinity pull-downs, indicate a specific and direct interaction between SUG1 and XPB in vivo.

Characterization of the hSUG1 complex

The direct and specific interaction between XPB and mSUG1 by the two-hybrid assay in yeast and the co-expression studies in insects cells do not prove that this interaction is of physiological significance. For instance, SUG1 may interact with an XPB domain not available when the latter is in a complex with TFIIH. Therefore, TFIIH from fractionated HeLa WCE (Fig. 5A) was tested for the presence of hSUG1 using MAb-SUG1 (2SU). Notably, SUG1 was detected in the Heparin SPW fractions 11 and 12, identical to the elution pattern of TFIIH (Fig. 5A). A physical association between hSUG1 and XPB was demonstrated by immunoprecipitations of Heparin SPW fraction 12 using MAbs against SUG1 (C- and N-terminal 2SU and 3SU, respectively). Both 2SU and 3SU specifically and efficiently depleted the fraction of hSUG1 (Fig. 5B, lanes 1–3). Moreover, in support of the two-hybrid and co-infection experiments, XPB, although not quantitatively depleted, also eluted from the SUG1 immunoprecipitations (Fig. 5B). To verify the specificity of the interaction the inverse immunoprecipitation was performed using MAb against XPB. Immunoprecipitates were washed with increasing salt concentrations prior to elution (Fig. 5C). Even after treatment with 1 M KCl salt, hSUG1 remained bound to the MAb XPB complex. However, immunoreactivity of SUG1 could not be found in the most pure (HAP) fraction of TFIIH (HAP is the final column of TFIIH purification), suggesting that SUG1 may dissociate during the preceding phenyl column.

To confirm the association of SUG1 with TFIIH in HeLa cells, we developed an alternative method for purification of TFIIH under more physiological conditions. A His6 and HA epitope was...
with proper functioning of the XBP protein, since both transfection and microneedle injection of the tagged cDNA encoding the double-tagged molecule was able to correct the repair-deficient XP-B cells (Winkler et al., manuscript submitted). The tagged XBP protein was not significantly overexpressed in the stable transformants used. WCE extracts were prepared from XPCS2BA (XP-B) cells expressing the functional tagged XBP and fractionated by nickel-chelate affinity chromatography and immunopurification using a 12CA5 MAb anti HA-tag. Material that bound specifically to both columns was eluted by competition with 100 mM imidazole or with a HA peptide, respectively. hSUG1 was present in both column eluates which were strongly enriched for TFIIH (Fig. 6). Similar results were obtained when a nucleolar extract was used to purify TFIIH, whereas a WCE from untagged HeLa cells did not contain detectable quantities of TFIIH subunits or SUG1 (data not shown). We determined that the HA column chromatography yields at least 1000-fold purification of TFIIH. In the reverse experiment, WCE extracts were prepared from HeLa cells expressing a HA-tagged mSUG1. Immunoblot analysis indicated that ~50% of the intracellular SUG1 is HA-tagged (data not shown). Both XBP and p62 were present in immunopurified HA-tagged mSUG1 complexes eluted with the HA peptide as analyzed by immunoblots (data not shown). In conclusion, these results clearly demonstrate that a fraction of SUG1 interacts with TFIIH in vitro in non-overexpressing human cells, however, the protein is not part of the final purified TFIIH complex that consists of at least nine subunits (XBP, XPD, p62, p52, p44, p34, cdk7, cyclin H and MAT1) (6).

The above experiments combined with its presence in the RNA polymerase II holocomplex (7,42,43,50), suggest that SUG1 may be directly or indirectly involved in transcription (45). To test this, we microinjected wild-type human diploid fibroblasts with a mSUG1 expression construct driven by a strong CMV promoter. At 24 h after injection to allow expression of the injected DNA, cells were pulse-labeled with [3H]uridine and processed for autoradiography. Dramatic morphological changes were observed in injected cells; the nuclei increased in size, the Giemsa-stainable chromatin material clumped into a small area and the cytoplasm became very densely stained. Concomitantly, transcription dropped eventually to zero compared to non-injected cells (Fig. 7A)
identified to suppress a GAL4 mutation in yeast cells, SUG1 has since been known to interact in a ligand-enhanced manner with nuclear receptors (39,45). Moreover, SUG1 has a direct interaction with TBP which was thought to be a target for its transcriptional mediation in yeast (43). Clearly, in the light of our findings, TFIIH may also be involved. Since the most pure TFIIH preparations lacking SUG1 are active in the in vitro transcription and repair reactions it can be concluded that SUG1 is apparently neither indispensable for the core of the basal transcription initiation mechanism nor for NER.

Functional implications of the SUG1–XPB interaction

The sequence identity between ySUG1 and mSUG1 is 74% indicative of a very strong sequence conservation throughout evolution. The most highly conserved region (88% identity) between the two proteins is a central domain of 244 amino acids (residues 132–375), abbreviated as the AAA module (for ATPases associated with a variety of cellular activities; 48). Proteins of the AAA family have been linked with numerous cell functions, including cell cycle regulation, secretion, vesicle-mediated transport, peroxisome biogenesis, gene expression and proteasome activity and are found not only in yeast and higher eukaryotes but also in archaeaacteria and euubacteria (48 and references therein). The members of this AAA protein family include: (i) the human proteasome component S7/MSS1 (52,53), also shown to modulate HIV Tat-mediated transcription, and the yeast homologue CIM5 (54); (ii) human TBP1 a protein that is closely related to TBP7 which binds the HIV Tat protein in vitro (55) and the yeast homolog YTA1 (56) (Fig. 1). Interestingly, the mSUG1 amino acid sequence is completely identical to a recently identified human subunit of the PA700 proteasome complex (44) and very closely related to the reported sequence of the human Trip1 protein. Trip1 was isolated in a two-hybrid screen in yeast using human Thyroid hormone receptor (TR-B1) as a bait (45).

What might be the functional implications of the SUG1–XPB interaction? SUG1 is a component of the 26S proteasome which has the capacity to interact with and degrade a diverse set of ubiquitinated proteins (27) but is also required for activation of proteins by processing inactive precursors (for reviews see 57–59 and references therein). The precise role of ATP hydrolysis in the mechanism of protein breakdown has not been identified, but may be linked to substrate unfolding and translocation into the proteolytic lumen of the 26S proteasome. To investigate the possibility that SUG1 bound to TFIIH is present in the proteasome form, we used proteasomal antibodies against S4 and MSS1 and showed that there is no immuneactivity in TFIIH fractions (Heparin 5PW, data not shown). Furthermore, using antibodies against ubiquitin we were unable to detect ubiquitinated forms of TFIIH. These findings render it unlikely that the fraction of TFIIH bound to SUG1 is undergoing proteolysis.

Alternatively, or in addition, SUG1 may act on its own or in a separate complex as an ATP-dependent molecular chaperone catalyzing protein conformational alterations (58). It is not excluded that SUG1 and the related ATPases MSS1 and TBP1 in this complex directly participate in the control of transcription (7,42,43,45,55).

Our findings demonstrate that only a small fraction of all cellular SUG1 is in a complex with TFIIH. This points to a more universal function for SUG1. Size-fractionation experiments of total cell extracts showed that the majority of SUG1 migrates over

DISCUSSION

mSUG1, the homolog of ySUG1, interacts with XPB

Our demonstration that SUG1 associates with TFIIH may explain its reported presence in the RNA polymerase II holoenzyme (7). However, in its active form, RNA polymerase II holocomplex does not appear to include SUG1 (27,50). The lowered affinity of SUG1 for the XPB bearing the XP11BE mutation, a mutation which lowers overall transcription levels, is consistent with the idea that SUG1 acts as a mediator of transcription (45). Originally

Figure 7. Effect of wild-type mSUG1 on transcription. Micrographs showing the effect of microinjection of wild-type mSUG1 encoding cDNA (pCMSUG1) on RNA synthesis in normal human fibroblasts. The injected polykaryons (indicated with an arrow) of which one nucleus is injected demonstrates a strong inhibition of RNA synthesis (A, B) and a clear chromatin collapse (B). Nuclei are indicated with arrowheads.

and B). These unusual effects are very similar to the injection of a dominant-negative XPB mutant (37) suggesting that SUG1 overexpression may act via the same mechanism as mutant XPB. No such phenomena were seen when numerous other wild-type genes on the same vector were injected such as XPB, XPD and p62. This experiment suggests that overexpression of mSUG1 in mammalian cells confers a dominant-negative effect on transcription in vivo. This is consistent with a direct or indirect role of SUG1 in transcription (51).
a broad but higher range than expected for free SUG1, indicating that this protein resides in (large) complexes (60). In glycerol gradients S4 and MSS1 peak with the PA700 while SUG1 is found throughout the gradient (40). These findings suggest that there might be a link between the transcription/repair and proteasome machineries.

The microinjection experiments support a role of SUG1 in transcription in vivo, probably by saturation of essential transcription components. In fact, besides other targets, XBP may be one of the factors that are saturated when SUG1 is overexpressed after microinjection of many SUG1 cDNA copies under a strong promoter.

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