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TFG3/TAF30/ANC1, a Component of the Yeast SWI/SNF Complex That Is Similar to the Leukemogenic Proteins ENL and AF-9

BRADLEY R. CAIRNS, N. LYNN HENRY, AND ROGER D. KORNBERG*

Department of Structural Biology, Stanford University School of Medicine, Stanford, California 94305

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The *SWI1*/*ADR6*, *SWI2*/*SNF2*, *SWI3*, *SNF5*, and *SNF6* gene products are all required for proper transcriptional control of many genes in the yeast *Saccharomyces cerevisiae*. Genetic studies indicated that these gene products might form a multiprotein SWI/SNF complex important for chromatin transitions preceding transcription from RNA polymerase II promoters. Biochemical studies identified a SWI/SNF complex containing these and at least six additional polypeptides. Here we show that the 29-kDa component of the SWI/SNF complex is identical to TFG3/TAF30/ANC1. Thus, a component of the SWI/SNF complex is also a member of the TFIIF and TFIID transcription complexes. TFG3 interacted with the SNF5 component of the SWI/SNF complex in protein interaction blots. TFG3 is significantly similar to ENL and AF-9, two proteins implicated in human acute leukemia. These results suggest that ENL and AF-9 proteins interact with the SNF5 component of the human SWI/SNF complex and raise the possibility that the SWI/SNF complex is involved in acute leukemia.

Many genetic and biochemical experiments have established an important role for chromatin in the repression of transcription (15). For example, a DNA molecule containing a binding site for a transcriptional activator may display a much lower level of activator occupancy when the binding site is packaged in a nucleosome (59). In addition, there is considerable evidence that many nonhistone chromatin components assist in the establishment or maintenance of chromatin structure and contribute to repression. Before transcription can occur, this repression must be overcome to make the DNA accessible to both activators and the basal transcription machinery. Such chromatin structural changes apparently occur at the yeast *PHO5* and *SUC2* promoters, where positioned nucleosomes are disrupted under derepressing growth conditions (20, 48). Importantly, this disruption requires neither DNA replication nor transcription from the promoter to occur (48). The observed chromatin transition thus precedes transcription and is not coupled to it.

Genetic studies from several laboratories identified five of the components of a multiprotein complex which may help mediate chromatin transitions. They include the products of the *SWI1*/*ADR6*, *SWI2*/*SNF2*, *SWI3*, *SNF5*, *SNF6*, and *SNF11* genes, all of which (except *SNF11*) are required for proper control of the same set of yeast promoters (13, 17, 32, 33, 40, 43, 54). Their action in a complex was inferred from experiments with LexA-SNF2 and LexA-SNF5 fusion proteins; both chimeric proteins required all five SWI and SNF proteins to activate transcription from a promoter containing a LexA upstream activating sequence (UAS). Additional genetic studies suggest that these proteins antagonize the repressive effects of chromatin (4, 58). For example, extragenic suppressors of both *swi* and *snf* mutants reveal mutations in genes encoding histones and nonhistone chromatin components (29, 51). In addition, the chromatin transitions that occur at the *SUC2* promoter in the absence of glucose repression in wild-type yeast cells do not occur in *snf2* or *snf5* mutants (20). Biochemical purification verified that these SWI and SNF proteins form a stable complex and demonstrated that a DNA-dependent ATPase activity associated with the SWI2/SNF2 component copurifies with the complex (3, 7, 42). Recent studies indicate that the yeast SWI/SNF complex utilizes ATP to perturb nucleosome structure and to assist GAL4 derivatives in their binding to nucleosomal DNA (7). Similar results have been obtained with highly purified fractions derived from human cells containing a complex with a homolog of SWI2/SNF2 (23, 30).

We and others have previously demonstrated that SWI/SNF complex contains several polypeptides in addition to those described above (3, 7). Here, we identify one of these polypeptides as TFG3, a protein which is also an integral member of two other yeast transcription factor complexes, TFIID and TFIIF.

MATERIALS AND METHODS

Yeast strains. A *Saccharomyces cerevisiae* strain lacking the TFG3 gene was prepared by one-step gene replacement with the knockout plasmid pPL2 and the parent strain YPH499 (*MAT***a** *his3-*Δ*200 trp1-*Δ*3 ura3-52 leu2 ade2-101 lys2-806*). The disruption plasmid pPL2 was prepared and cut as described previously (18). The *tfg3*Δ::*LEU2*/*TFG3* heterozygous diploid strain is a derivative of W303 and was prepared as described previously (18). The *swi1*Δ strain CY58 was a gift of Craig Peterson, University of Massachusetts Medical Center, Worcester, Mass., and is congenic to YPH499.

Purification of the SWI/SNF complex. The SWI/SNF complex was purified from two strains, BJ926 and Fleischmann's active yeast (gift of Fleischmann's Yeast Inc., Oakland, Calif.). Extractions from 12 kg of cells and the first three chromatographic steps (Bio-Rex 70, DEAE-Sephacel, and hydroxylapatite) were performed as described previously (47). Peak fractions from hydroxylapatite were further resolved on Mono Q. Adsorbed proteins were eluted with a linear gradient of 200 to 1,000 mM potassium acetate in buffer A (20 mM Tris-acetate [pH 7.6], 20% glycerol, 1 mM dithiothreitol, 1 mM EDTA, 2 μg of chymostatin per ml, 2 μM pepstatin A, 0.6 μM leupeptin, 2 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 0.01% Nonidet P-40). Peak fractions of the SWI/SNF complex eluting at 750 mM potassium acetate were diluted twofold in buffer A lacking potassium acetate, bound batchwise for 4 h at 4°C to an SNF6 antibody immunoaffinity resin (2.5-ml bed volume) (described below), washed in a column with 50 ml of buffer A containing 700 mM potassium acetate and 0.2% Nonidet P-40, and eluted with 5 M urea. The purification was monitored by immunoblot analysis with antisera to SNF6 and SWI3.

The complexes derived from the two cell sources had identical polypeptide compositions and were therefore combined to obtain peptide sequence information. To the combined 5 M urea eluates (8 ml) were added 120 μg of insulin, deoxycholate to a final concentration of 0.2%, and trichloroacetic acid to a final concentration of 10%. After incubation for 1 h at 4°C and centrifugation at 13,000 × *g* for 20 min at 4°C, the resulting pellets were washed with 200 μl of acetone at 4°C, resuspended in 2× sodium dodecyl sulfate (SDS) loading buffer, and heated at 65°C for 2 min. Approximately 200 μg of SWI/SNF complex was

* Corresponding author. Phone: (415) 723-6988. Fax: (415) 723-8464.

separated in a single lane of an SDS–9% acrylamide gel and transferred for 12 h at 10 V/cm to a polyvinylidene difluoride membrane (Bio-Rad Trans Blot) in a transfer buffer containing 25 mM Tris (pH 8.3), 192 mM glycine, 10% methanol, and 0.005% SDS. Proteins were revealed by staining with Ponceau S and excised. Peptide sequencing was performed by the Harvard Microchemistry Facility, Cambridge, Mass.

An alternative procedure was used to purify the complex to near homogeneity without the use of an immunoaffinity column. Peak fractions from the Mono Q column were pooled, dialyzed against buffer B (20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.6], 20% glycerol, 1 mM dithiothreitol, 1 mM EDTA, 2 μg of chymostatin per ml, 2 μM pepstatin A, 0.6 μM leupeptin, 2 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 0.01% Nonidet P-40), containing 100 mM potassium acetate, applied to a TSK-heparin column, and eluted in buffer B with a linear gradient of 200 to 800 mM potassium acetate. Peak fractions (from 450 to 490 mM potassium acetate) were pooled, dialyzed against buffer B, applied to a Mono S column, and eluted in buffer B with a linear gradient of 200 to 800 mM potassium acetate. Peak fractions (from 350 to 380 mM potassium acetate) were pooled, dialyzed against buffer A containing 100 mM potassium acetate, applied to a DEAE-Sephacel column, and eluted in buffer A with a linear gradient of 200 to 800 mM potassium acetate. The peak of the SWI/SNF complex eluted at 470 mM potassium acetate. Because of the presence of contaminants eluting at 400 to 450 mM potassium acetate, fractions preceding the peak were about 25 to 40% pure and fractions after the peak were about 40 to 70% pure, as determined by electrophoretic separation and staining with Coomassie blue dye.

Immunoprecipitation and immunodepletion experiments. Crude SNF6 and affinity-purified TFG3 antiserum were coupled to protein A-Sepharose as described previously (3). To prepare anti-SNF6 and anti-TFG3 immune complexes, the peak SWI/SNF fraction from the Mono S column (25 μl, 15 μg) was first diluted twofold in buffer A lacking potassium acetate and precleared with 20 μl of 50% protein A-Sepharose beads (in buffer A containing 0.1 M potassium acetate). Samples were sedimented in a microcentrifuge, and the supernatants were incubated for 3 h at 4°C with either 20 μl of 50% protein A-Sepharose beads coupled to anti-SNF6 or 20 μl of 50% protein A-Sepharose beads coupled to anti-TFG3. The beads were sedimented in a microcentrifuge, and the resulting "IP" supernatant was conserved, while immune complexes on the beads were washed three times with 500 μl of buffer A containing 600 mM potassium acetate and 0.2% Nonidet P-40 and eluted twice with 20 μl of 5 M urea.

To prepare anti-SNF6 immune complexes with SWI/SNF lacking TFG3, the peak fraction from Mono S (60 μl, 40 μg; <5% SWI/SNF complex) was precleared with 50 μl of 50% protein A-Sepharose beads, incubated for 4 h at 4°C with 50 μl of protein A-Sepharose beads conjugated to anti-SNF6 antibodies, centrifuged, and the supernatant (120 μl) was saved. Immune complexes were washed three times with 1 ml of buffer A containing 500 mM potassium acetate and 0.2% Nonidet P-40 and were boiled for 5 min in 40 μl of SDS loading buffer. To load half of the IP supernatant in a single lane of an SDS-containing gel, the material was precipitated with deoxycholate (0.2%) and trichloroacetic acid (10%).

Coimmunoprecipitation assays with SWI/SNF complex and TFIIF lacking TFG3. TFIIF lacking TFG3 (TFIIF*) was purified as described previously (18). Highly purified SWI/SNF complex from DEAE-Sephacel was dialyzed against buffer A containing 100 mM potassium acetate, and 20 μl of this fraction (6 μg; 40% SWI/SNF complex) was precleared with 25 μl of 50% protein A-Sepharose (in buffer A containing 100 mM potassium acetate) by rocking for 1 h at 4°C. The sample was centrifuged, and the supernatant (40 μl) was mixed with 20 μl (50 ng) of near-homogeneous TFIIF* (in buffer A [400], where [400] indicates the millimolar salt concentration). The sample was diluted to a final volume of 100 μl with buffer A [100], creating a potassium acetate concentration of 180 mM. The sample was rocked at 4°C for 90 min, and then 40 μl of 50% protein A-Sepharose beads conjugated to SNF6 antibody (in buffer A [100]) was added, creating a final potassium acetate concentration of 160 mM. The sample was rocked for 4 h at 4°C and centrifuged briefly in a microcentrifuge at 4,000 × *g*, and the supernatant was removed and stored in liquid nitrogen. The pellets were washed twice with 1 ml of buffer A [200] and once with 1 ml of buffer A [500] and then eluted with two 25-μl aliquots of 5 M urea.

Antibodies. Polyclonal antisera to SNF6 and SNF5 were prepared as previously described (3). Polyclonal antiserum to SWI3 was a gift of Craig Peterson. Affinity-purified TFG3 antiserum was prepared as previously described (18).

Immunoblot analyses. All immunoblots were incubated for 8 h with a 1:500 dilution of primary antibody in TBS–2% milk for 8 h and then for 1 h with a 1:2,000 dilution of goat anti-rabbit secondary antibody (Bio-Rad) conjugated to alkaline phosphatase. Immunoblots were developed as described previously (3).

Plasmids. Plasmids directing the expression of LexA fusion proteins are all derivatives of pSH2-1 (*HIS3*, 2μm origin), which directs the expression of LexA 1–87 from the *ADH1* promoter (a gift of S. Hanes). Plasmids pLEXA-SNF2, pLEXA-SNF5, pLEXA-SNF6 (gifts of B. Laurent), and pLEXA-GAL4 (pSH17-4; a gift of S. Hanes) have been described previously (16, 32, 33). Plasmid pLEXA-GRact (pGNLX, *TRP1*, 2μm origin; a gift of K. Yamamoto) directs expression of the first 452 amino acids of the rat glucocorticoid receptor fused to amino acids 1 to 87 of LexA from the yeast *GPD* promoter (60). Plasmid pGAL4-TFG3 contains the first 147 amino acids of GAL4 fused to the entire *TFG3* coding sequence. It was prepared by creating an *Eco*RI site at the 5′ end and a *Bam*HI site at the 3′ end of the *TFG3* gene by PCR with *Taq* polymerase, plasmid pPL2 (a genomic subclone of *TFG3*), and the oligonucleotide primers 5′-ATATGAATTCGTAGCGACAGTAAAAAGAACCATC-3′ and 5′-ATATGGATCCTTACTCGGTATTTTTCTT-3′. The 5′ oligonucleotide primer includes the three amino acids encoded by the first exon of *TFG3* and directs amplification from the second exon. Amplified DNA products were digested with *Eco*RI and *Bam*HI and cloned into plasmid pGBT9 (2μm origin, *TRP1*), which directs expression of GAL4 1–147 from the yeast *ADH1* promoter (a gift of P. Bartel and S. Fields, State University of New York, Stony Brook, N.Y.). The DNA sequence flanking the *Eco*RI junction was confirmed by DNA sequencing. Plasmid pLEXA-TFG3 was prepared by cloning the *Eco*RI-*Bam*HI fragment from pGAL4-TFG3 into the corresponding sites in pSH2-1.

β-Galactosidase assays. Transcriptional activation in vivo was quantified by β-galactosidase assays with the reporter plasmid pRS1840 (2μm origin, *URA3*), which contains a single high-affinity binding site for the *Escherichia coli* repressor LexA (a gift of S. Hanes). Strains containing pRS1840 (alone or in combination with plasmids expressing LexA fusion proteins) were grown to an optical density at 600 nm of 0.5 to 0.8 in 100 ml of synthetic medium containing the appropriate amino acids and 2% glucose. The cell suspension was centrifuged at 5,000 × *g* for 10 min, washed with sterile water, harvested, and suspended in 0.5 ml of buffer A containing 400 mM sodium chloride. The cells were disrupted by beating with glass beads for 9 min at 4°C. The extract was centrifuged at 13,000 × *g* for 10 min, and the supernatant was centrifuged again at 13,000 × *g* for 10 min. β-Galactosidase activities, determined as described previously (3), are given in units per milligram of protein in the whole-cell extract.

Far Western (protein-blot) analyses. A DEAE-Sephacel fraction (4 μg; approximately 70% SWI/SNF complex) and 1 μg of homogeneous TFIIF were separated on an SDS–8% acrylamide gel, transferred to nitrocellulose, and renatured as described by Vinson et al. (65). The filter was incubated overnight at 4°C in hybridization buffer (20 mM HEPES [pH 7.6], 75 mM KCl, 0.1 mM EDTA, 2.5 mM $MgCl_2$, 1 mM dithiothreitol, 0.05% Nonidet P-40, 1% dry milk), probed with 5 μl (10% of the in vitro transcription-translation mixture [Promega Inc.]) of ^{35}S-labelled full-length TFG3 protein, washed with hybridization buffer, and exposed to XAR film for 5 days.

RESULTS

Large-scale purification of the yeast SWI/SNF complex. The yeast SWI/SNF complex was purified from whole-cell extracts by three ion-exchange steps followed by anti-SNF6 immunoaffinity chromatography. The anti-SNF6 immunoaffinity column was washed extensively with a buffer containing 700 mM potassium acetate and nonionic detergent. The eluate of this column, in 5 M urea, contained at least 11 polypeptides; under these elution conditions, SNF6 remained bound to the column (Fig. 1). Immunoblot analysis confirmed that four of these polypeptides were SWI1/ADR6, SWI2/SNF2, SWI3, and SNF5 (results not shown). One additional polypeptide with an apparent molecular mass of 20 kDa, encoded by the *SNF11* gene, is observed only when the SDS-polyacrylamide gel is stained with Coomassie blue dye (64). Previous work has identified four additional polypeptides (SWP59, SWP61, SWP73, and SWP82) as members of the SWI/SNF complex and three other polypeptides (p29, p57, and p77) as possible members (3, 7). Protein p29 was present in all preparations.

p29 is identical to TFG3/ANC1. The polypeptides from approximately 200 μg of SWI/SNF complex were separated by SDS-polyacrylamide gel electrophoresis (PAGE), transferred to a synthetic membrane, excised, and treated with a protease. The following high-confidence sequence from p29 was obtained: IEEQGWGGFPLDISVF. An identical sequence of residues is present in the TFG3 protein at positions 76 to 91.

We have previously reported that TFG3 is an integral component of both yeast TFIID and yeast TFIIF, two factors required for basal transcription by RNA polymerase II (18, 19). Yeast TFIIF is a complex of three proteins: TFG1, TFG2, and TFG3. TFG1 and TFG2 are significantly similar to the mammalian Rap74 and Rap30 proteins, respectively, which form mammalian TFIIF (14, 18, 22). Both yeast TFIIF and mammalian TFIIF form a complex with RNA polymerase II, and each is required for polymerase II transcription in vitro (18, 36). *TFG1* and *TFG2* are essential genes (18). In contrast, TFG3 protein is at most stimulatory to transcription, and de-

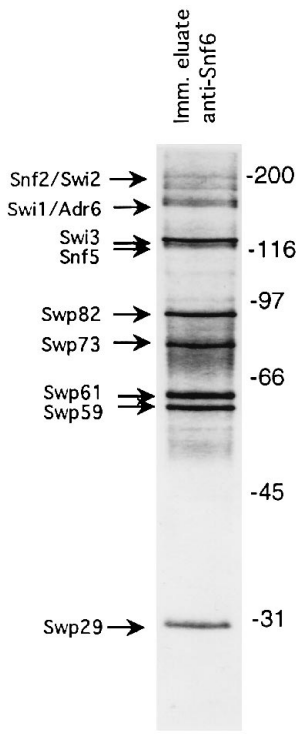

FIG. 1. Purified SWI/SNF complex contains a 29-kDa protein. The SWI/SNF complex was purified on a large scale by the immunoaffinity procedure as described in Materials and Methods. The eluate from the anti-SNF6 immunoaffinity column (5 μg) was separated in a SDS–10% acrylamide gel and stained with Coomassie blue dye.

letion of the *TFG3* gene confers a temperature-sensitive phenotype (18, 56). Yeast TFIIF is known to form a stable complex with RNA polymerase II holoenzyme, which is a combination of RNA polymerase II, SRB proteins, GAL11, and several other polypeptides; this complex enables a response to activators (27). *ANC1*, which is identical to *TFG3*, was isolated by Welch et al. in a screen for mutants which fail to complement a temperature-sensitive allele of actin (57). However, TFG3 protein resides exclusively in the nucleoplasm, which is devoid of actin, further suggesting a role for TFG3 in transcriptional regulation (see Discussion) (56).

Immune complexes formed with anti-TFG3 antisera contain all other members of the SWI/SNF complex. Further evidence that TFG3 is a member of the SWI/SNF complex was obtained by immunoprecipitation and immunoblot analyses. SWI/SNF complex devoid of TFIIF or TFIID was prepared for this purpose. Following three ion-exchange steps, chromatography on Mono Q yielded SWI/SNF complex free of mediator and TFIID (determined by immunoblot analyses with anti-TBP, anti-SRB4, and anti-SRB5 antibodies) but containing detectable amounts of TFIIF. Mono S chromatography then separated the SWI/SNF complex from TFIIF. After removal of TFIIF, TFG3 cofractionated precisely with the other members of the SWI/SNF complex. Likewise, during further fractionation of TFIIF, TFG3 cofractionated precisely with the other components of TFIIF (data not shown).

The peak Mono S fraction (approximately 20% SWI/SNF complex) was immunoprecipitated with affinity-purified anti-TFG3 antibodies coupled to protein A-Sepharose and then extensively washed with a buffer containing 700 mM potassium acetate and 0.2% Nonidet P-40. SDS-PAGE analysis and staining with silver revealed only the other members of the SWI/SNF complex (Fig. 2A). Although the immunoaffinity column was eluted with 5 M urea, the antigen (TFG3) remained bound to the antibody. In a converse experiment, SDS-PAGE and immunoblot analysis of immune complexes prepared with the peak Mono S fraction and anti-SNF6 antibodies revealed a protein with an apparent molecular mass of 29 kDa that comigrated with the TFG3 component of yeast TFIIF (Fig. 2B and C, respectively). Other members of the SWI/SNF complex such as SNF6 and SWI3 were fully depleted from the Mono S fraction by anti-TFG3 antibodies, showing that all SWI/SNF complexes in the fraction contained TFG3 (Fig. 2D). In addition, we have determined that TFG3 is not a component of other yeast transcription factor complexes such as TFIIE or TFIIH. We conclude that TFG3 is not simply an abundant protein with a high nonspecific affinity for proteins but, rather, an integral component of multiple complexes.

Deletion of TFG3 does not confer many of the phenotypes associated with SWI/SNF complex. Mutations in or deletions of either *SWI1*/*ADR6*, *SWI2*/*SNF2*, *SWI3*, *SNF5*, or *SNF6* confer similar phenotypes, including slow growth and defects in transcriptional activation of several genes including *SUC2* (encoding invertase) and *HO* (encoding an endonuclease required for mating-type switching) (5, 39, 50, 58). Under anaerobic conditions, *swi* and *snf* mutants grow very poorly on solid medium containing either sucrose, galactose, or raffinose (Snf^- phenotype). Anaerobic conditions can be mimicked by the addition of antimycin A (1 μg/ml; an inhibitor of electron transport in the respiratory chain) to the medium. This treatment dramatically reduces the growth rate of *swi* and *snf* mutants on sucrose, galactose, or raffinose but has only a modest effect on their growth on glucose (39).

Welch and Drubin (56) have shown that *tfg3*/*anc1* mutants are temperature sensitive (Ts^-), but a possible Snf^- phenotype was not reported. We prepared a strain lacking the *TFG3* gene and tested it for certain phenotypes and defects characteristic of *swi* and *snf* mutants. As observed with such mutants, the *tfg3*Δ strain grew slowly at 30°C on solid media lacking antimycin A and containing 2% glucose, compared with its wild-type parent. Several of the phenotypes displayed by the *tfg3*Δ strain, however, contrasted with phenotypes reported for mutants in other SWI/SNF complex components. For example, the presence of 1 μg of antimycin A per ml had little effect on the growth of *tfg3*Δ cells on solid rich medium containing glucose, sucrose, galactose, or raffinose at either the permissive (30°C) or semipermissive (34°C) temperature. In addition, the *tfg3*Δ strain grew more poorly in galactose than in sucrose, a relative difference not observed with either the wild-type parent or other *swi* and *snf* mutants. To ensure that the Snf^+ phenotype was not due to a reversion of our haploid *tfg3*Δ strain, we observed the segregation pattern of the Snf phenotype in eight tetrads derived from our *tfg3*Δ::*LEU2*/*TFG3* heterozygous diploid strain. Each of the eight four-spore tetrads tested produced two Leu^+ Ts^- Snf^+ spores and two Leu^- Ts^+ Snf^+ spores. These results show that the lack of *TFG3* does not cause a Snf^- phenotype. Finally, in contrast to a congenic *swi3*Δ strain, the *tfg3*Δ strain will not form colonies at 37°C on rich media containing glucose.

Additional differences between the *tfg3*Δ strain and strains lacking other SWI and SNF components were observed in transcriptional activation (Table 1). Others have shown that fusion of the DNA-binding domain of the bacterial repressor LexA (amino acids 1 to 87) to SNF2, SNF5, SNF6, GAL4, or the activation domain of the rat glucocorticoid receptor (GRact) creates fusion proteins that are potent activators when assayed

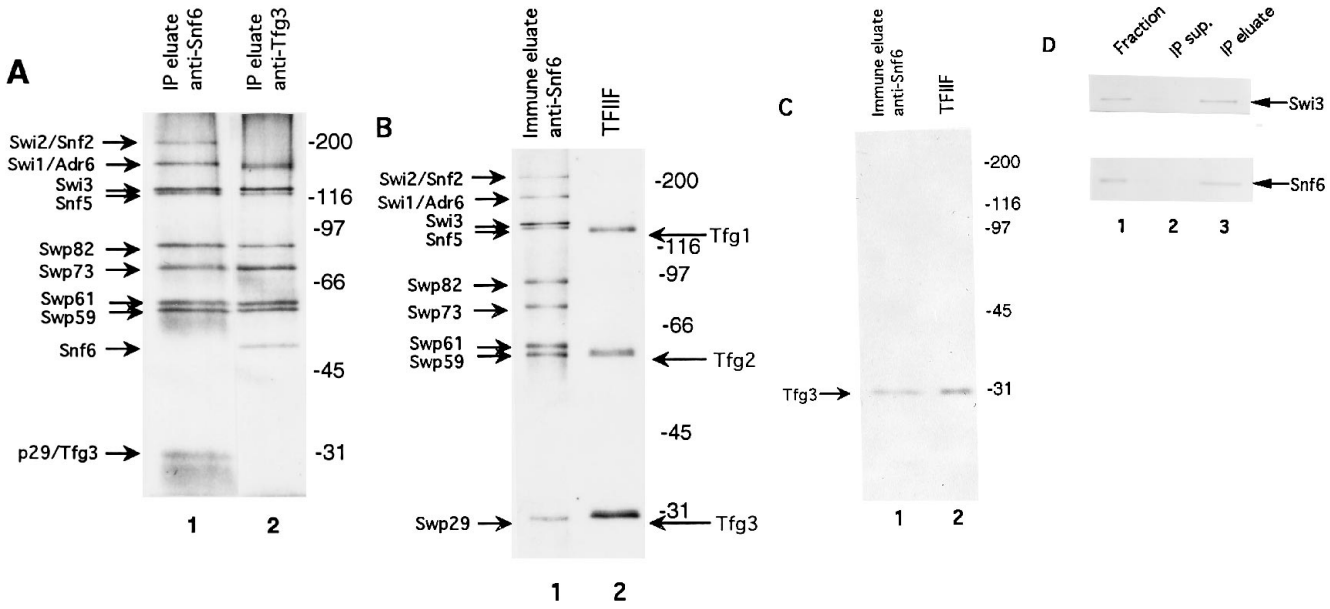

FIG. 2. Analysis of anti-SNF6 and anti-TFG3 immune complexes with SDS-PAGE and immunoblots. Immune complexes were formed with either anti-SNF6 or anti-TFG3 antibody conjugated to protein A-Sepharose and the peak Mono S fraction (20 μg; <5% SWI/SNF complex) as described in Materials and Methods. (A) Half of the immune eluate from anti-SNF6 (lane 1) or anti-TFG3 (lane 2) immune complexes was separated on an SDS–10% acrylamide gel and stained with silver. Since the immunoprecipitations are performed without reducing agents (to lower the background of antibody released), the SWI2/SNF2 protein gradually becomes linked to the immunoaffinity column and can be detected in the immune pellet after other SWI/SNF components have been eluted with 5 M urea (data not shown). (B) Analysis of the anti-SNF6 immune eluates (lane 1) and homogeneous TFIIF (lane 2) by SDS-PAGE and silver staining. (C) Immunoblot analysis of TFIIF and the SWI/SNF complex with affinity-purified anti-TFG3 antisera. Homogeneous TFIIF (100 ng; lane 2) and one-fifth of the immune eluate from the anti-SNF6 immunoprecipitation (lane 1) were separated on an SDS–10% acrylamide gel, immunoblotted, and probed with affinity-purified anti-TFG3 antisera. (D) Anti-TFG3 antibodies can immunodeplete members of the yeast SWI/SNF complex. The untreated Mono S fraction (3.0 μg; lane 1), one-fifth of the IP supernatant (3.0 μg; lane 2), and two-fifths of the anti-TFG3 immune eluate (lane 3) were separated on an SDS–10% acrylamide gel, immunoblotted, and probed with either anti-SNF6 or anti-SWI3 antiserum.

with a β-galactosidase reporter plasmid containing a single LexA-binding site (16, 31–33, 60). These studies demonstrated that the previously identified components of the SWI/SNF complex are required for these hybrid proteins to activate at full capacity.

We found that LexA-GRact fusion protein was a potent activator in both wild-type and *tfg3*Δ strains but did not activate transcription in the congenic *swi1*Δ strain. In addition, both LexA-SNF2 and LexA-SNF5 fusion proteins activated transcription at full capacity in the *tfg3*Δ strain but no activation was observed in a *swi1*Δ strain. To ensure that this observation was not due to a reversion in our *tfg3*Δ strain, we transformed LexA-SNF2 into four strains derived from one tetrad of our heterozygous *tfg3*Δ::*LEU2*/*TFG3* diploid strain. LexA-SNF2 was a potent activator in all four strains, further demonstrating that activation by LexA-SNF2 does not require *TFG3*. Unlike LexA-SNF2 or LexA-SNF5, LexA-SNF6 is reported not to require other components of the complex to activate transcription (33). We observed that LexA-SNF6 activated transcription at reduced capacity in the *tfg3*Δ strain compared with the wild-type strain. Immunoblot analysis with anti-SNF6 antibodies revealed, however, that at least 10-fold less LexA-SNF6 protein was present in the *tfg3*Δ strain than in the wild-type cells, which may account for our observation (data not shown). Finally, LexA-GAL4 is a potent activator in the wild-type strain but did not activate significantly in either *tfg3*Δ or *swi1*Δ cells. Again, we assessed LexA-GAL4 expression by immunoblot analysis and determined that at least 20-fold less LexA-GAL4 protein is present in *tfg3*Δ transformants than in wild-type transformants (data not shown). We have previously reported that the activation observed with a reporter plasmid containing a single GAL4-binding UAS element is reduced approximately sixfold in *tfg3*Δ cells (18). Thus, the dramatic reduction in activation observed with LexA-GAL4 may be explained in part by a combination of these effects. These results demonstrate that (in contrast to strains lacking SWI1, SWI2/SNF2, SWI3, SNF5, or SNF6 proteins) TFG3 protein is not required for certain activators to operate at full capacity. In addition, the activators tested that displayed a reduced capacity to activate transcription are correspondingly present at reduced levels in *tfg3*Δ cells.

TABLE 1. Potency of LexA-activator fusion proteins assessed in β-galactosidase assays[a]

Activator expressed	β-Galactosidase activity (U/mg) of:		
	Wild type	*tfg3*Δ	*swi1*Δ
LexA-1-87 (control)	12	8	ND
LexA-SNF2	2,039	1,651	5
LexA-SNF5	2,353	1,980	4
LexA-SNF6	8,300	1,124	127
LexA-GRact(1–452)	1,863	3,733	2
LexA-GAL4	6,200	32	65

[a] The indicated LexA fusion proteins were expressed and β-galactosidase assays were performed as described in Materials and Methods. Transformants also contained the reporter pRS1840, which contains one 22-bp LexA operator upstream of the minimal GAL1 promoter fused to the β-galactosidase gene. The values reported are the average of at least four transformants.

All *swi* mutants are defective in the transcription of the *HO* gene, which encodes an endonuclease required for mating-type switching. To assess the extent to which TFG3 is required for *HO* activation, we assessed the UAS activity of the SWI5/

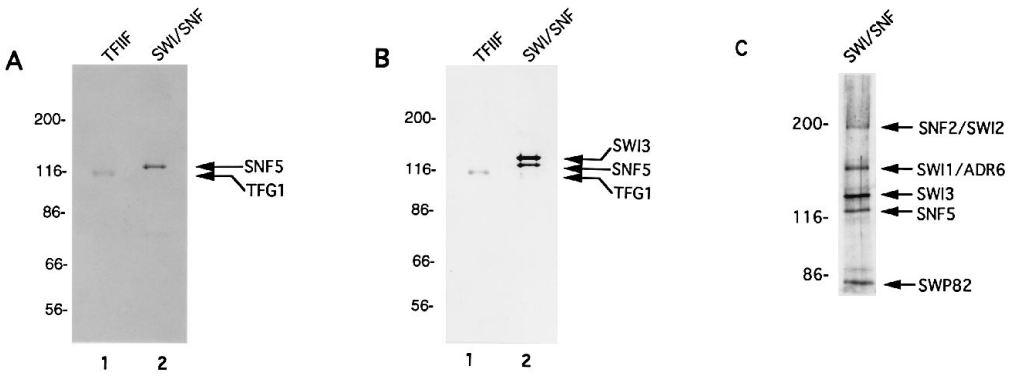

FIG. 3. TFG3 interacts with SNF5 in a far Western analysis. (A) Far Western analysis with ^{35}S-labelled TFG3 protein and renatured TFIIF or SWI/SNF complex. Homogeneous TFIIF (1 μg, lane 1) and a DEAE-Sephacel fraction (4 μg, 70% SWI/SNF complex; lane 2) were separated on an SDS–8% acrylamide gel, transferred to nitrocellulose, and renatured. The filter was probed with ^{35}S-labelled full-length TFG3 protein, washed with hybridization buffer, and exposed to XAR film for 5 days. (B) The nitrocellulose filter was probed and developed sequentially with affinity-purified antiserum to TFG1, polyclonal antiserum to SNF5, and affinity-purified antiserum to SWI3. (C) SDS-PAGE analysis of the DEAE-Sephacel fraction. The DEAE-Sephacel fraction (2 μg; 70% SWI/SNF complex) was separated on an SDS–6% acrylamide gel and stained with silver.

PHO2(GRF10) element from the *HO* promoter (carried on plasmid M632) in wild-type and *tfg3*Δ cells (2). We have observed a 10- to 20-fold reduction in β-galactosidase levels in certain *swi* and *snf* mutant cells containing this reporter relative to wild-type cells (2a). Extracts from wild-type cells contained 14 U of activity compared with 10 U observed from *tfg3*Δ cells, indicating that deletion of *TFG3* has little effect on activation with this element.

A LexA-TFG3 fusion protein is only a weak activator. To further investigate the role of TFG3 in transcriptional activation, we fused the DNA-binding domain of GAL4 (amino acids 1 to 147) or of LexA (amino acids 1 to 87) to the amino terminus of TFG3. The resulting vectors, pGAL4-TFG3 and pLEXA-TFG3, contained a high-copy 2μm origin and the strong constitutive *ADH1* promoter. Expression of LexA-TFG3 in *tfg3*Δ cells fully complemented their growth defect at 30°C and their temperature sensitivity at 37°C, whereas expression of the GAL4-TFG3 fusion protein in *tfg3*Δ cells only partially complemented these defects. Immunoblot analysis with anti-TFG3 antibodies showed that GAL4-TFG3 protein was expressed at slightly higher levels in wild-type than *tfg3*Δ transformants and that this level was approximately fivefold lower than the amount of endogenous TFG3 protein in wild-type cells (data not shown).

The ability of these TFG3 fusion proteins to activate transcription was assessed with β-galactosidase reporter plasmids containing a single binding site for either GAL4 or LexA as the UAS. GAL4-TFG3 protein activated transcription only weakly in both wild-type and *tfg3*Δ cells. Extracts prepared from wild-type or *tfg3*Δ cells expressing GAL4-TFG3 contained only 112 or 86 U of activity, respectively, compared with 5 U obtained with the reporter plasmid alone. Extracts prepared from wild-type or *tfg3*Δ cells expressing LexA-TFG3 contained only 35 or 29 U of activity, respectively, compared with 6 U obtained with a control reporter plasmid. The lack of strong activation by either LexA-TFG3 or GAL4-TFG3 may be a result of more than one factor. First, either alone or as a member of a complex, they may repress or interfere with activation. Second, these proteins may not interact well with the SWI/SNF complex or may be sequestered in the more abundant TFIID or TFIIF complexes. Finally, since LexA-SWI3 is only a weak activator, although it will fully complement a SWI3 mutant (54), not all fusions to SWI/SNF complex members necessarily create potent activators.

TFG3 interacts with the SNF5 component of the SWI/SNF complex in a far Western analysis. To determine which component(s) of the SWI/SNF complex interacts with TFG3, protein blots of the complex were probed with ^{35}S-labelled TFG3 protein. A fraction derived from DEAE-Sephacel (approximately 70% pure SWI/SNF complex), along with a fraction of homogeneous TFIIF, was separated by SDS-PAGE. The proteins were transferred to nitrocellulose, renatured, and probed with ^{35}S-labelled TFG3 protein. Autoradiography revealed an interaction of TFG3 with a protein of approximately 110 kDa in TFIIF and with a protein of approximately 120 kDa in the SWI/SNF complex (Fig. 3A). We have shown previously that the 110-kDa component of TFIIF is TFG1 and that the SWI/SNF complex contains two proteins, SWI3 and SNF5, that migrate on SDS-PAGE at approximately 120 kDa (3, 18). To clarify the identity of the protein interacting with TFG3, the blot was probed and developed sequentially with affinity-purified antiserum to TFG1, polyclonal antiserum to SNF5, and affinity-purified antiserum to SWI3 (Fig. 3B). Superimposition of the autoradiograph with the immunoblot clearly revealed SNF5 as the interacting protein. By performing the immunoblot analysis in this order, we also verified that the DEAE-Sephacel fraction utilized did not contain any TFG1 protein. Electrophoresis of the fraction in a 6% acrylamide–SDS gel followed by staining with silver revealed no additional polypeptide migrating near SNF5 (Fig. 3C). To help clarify the region responsible for these interactions, a truncation of TFG3 that removed 88 amino acids from the carboxy terminus (TFG3ΔC) was prepared. ^{35}S-labelled TFG3ΔC failed to interact with either SNF5 or TFG1, indicating that this region is required for these interactions or for proper protein folding.

If TFG3 utilizes the same domain(s) for binding to SNF5 as for interaction with TFG1, SNF5 and TFG1 might contain regions of homology. To test this possibility, we performed a rigorous comparison of the protein sequences of SNF5 and TFG1 with the program PEP-Align (IntelliGenetics). No significant regions of homology were detected, indicating that TFG3 does not physically interact with identically positioned residues in both SNF5 and TFG1.

Activation of the DNA-dependent ATPase does not cause the SWI/SNF complex to disassemble, or to assemble with TFIIF or RNA polymerase II holoenzyme in vitro. Our observation that TFG3 is a member of three distinct complexes involved in transcriptional activation raises the possibility that TFG3 pro-

vides a physical link between these complexes. To investigate this possibility, we tested whether TFG3 displays homotypic interactions. ^{35}S-labelled TFG3 failed to interact with TFG3 protein derived from the SWI/SNF complex in far Western analyses (data not shown). Furthermore, no significant binding of either homogeneous TFIIF or nearly homogeneous RNA polymerase II holoenzyme (a complex of core RNA polymerase II and mediator complex, containing TFIIF and other proteins) to immunoprecipitates of the SWI/SNF complex was observed under either moderate- or high-ionic-strength conditions (160 to 180 mM or 500 mM potassium acetate, respectively). These potential interactions are not enabled by activation of the SNF2/SWI2 DNA-dependent ATPase, since prior incubation of these factors and SWI/SNF immunoprecipitates with Mg^{2+}, ATP, and DNA under conditions of low ionic strength (75 mM potassium acetate) did not effect an association stable to moderate- or high-ionic-strength conditions.

Another possible reason for the presence of TFG3 protein in multiple complexes is that it masks or protects a region used for interactions between these complexes. According to this idea, a conformational change by or the dissociation of TFG3 from the SWI/SNF complex may uncover a site of interaction. To test this idea, we investigated whether activation of the DNA-dependent ATPase would cause the dissociation of TFG3 (or other components) from the remainder of the complex. Immune complexes were formed with the peak Mono S fraction and either anti-SNF6 or anti-TFG3 coupled protein A-Sepharose beads. The immune precipitates were washed extensively and supplemented with a buffer and other components previously shown to be optimal for the DNA-dependent ATPase activity (3). None of the components of the SWI/SNF complex were released from either anti-SNF6 or anti-TFG3 immune complexes, indicating that either the activation of the DNA-dependent ATPase is not coupled to complex disassembly or only a small percentage of the complexes in this assay were involved in producing the observed DNA-dependent ATPase activity (data not shown).

A stable complex containing many SWI and SNF components is present in extracts from *tfg3*Δ cells. To determine whether a SWI/SNF complex could assemble in *tfg3*Δ cells, we monitored purification of the complex form *tfg3*Δ cells by immunoblot analyses with antisera against SWI2/SNF2, SWI3, SNF5, and SNF6 proteins. All four of these proteins copurified in the four chromatographic steps used (data not shown). Immunoprecipitation of the peak Mono Q fraction with anti-SNF6 antibodies coupled to protein A-Sepharose immunodepleted SWI2/SNF2, SWI3, SNF5, and SNF6 (Fig. 4A). Although the immunoprecipitate was extensively washed with a buffer containing 500 mM potassium acetate and 0.2% Nonidet P-40, all four proteins remained associated. These results demonstrate that a stable complex consisting of at least SWI2/SNF2, SWI3, SNF5, and SNF6 proteins can be isolated from *tfg3*Δ cells. These results also indicate that TFG3 is not required for SNF5 to stably interact with other components of the complex.

TFIIF from a *tfg3*Δ strain does not interact stably with the SWI/SNF complex from wild-type cells. If separate domains of TFG3 are responsible for interaction with the SWI/SNF complex and TFIIF, a single TFG3 molecule might tether the two complexes together. To test this possibility, we purified TFIIF to near homogeneity from *tfg3*Δ cells, isolating a complex of two proteins, TFG1 and TFG2 (TFIIF*). Interaction of TFIIF* with the SWI/SNF complex was assessed by coimmunoprecipitation with anti-SNF6 antibodies. No evidence of stable interaction was obtained; under conditions of moderate ionic strength (160 mM potassium acetate), TFIIF* was not immunodepleted with anti-SNF6 antibodies, nor was TFIIF* detected in the immune precipitate. The SWI/SNF complex was, however, efficiently precipitated, as evidenced by the lack of SWI3 protein in the IP supernatant and its presence in the precipitate (Fig. 4B). Identical results were obtained when the reactions were performed either with a fourfold excess of SWI/SNF complex over TFIIF* or with stoichiometric amounts of TFIIF* or wild-type TFIIF.

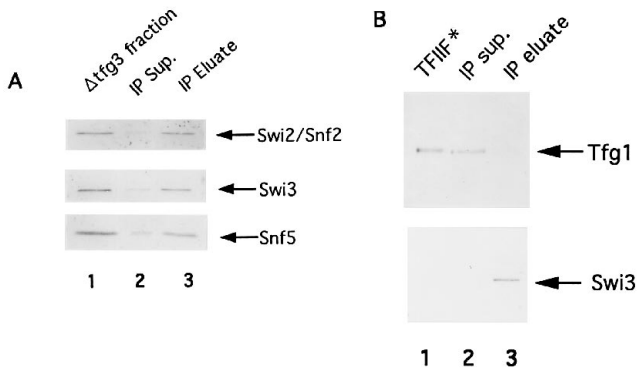

FIG. 4. The composition and interactions of the SWI/SNF complex and TFIIF purified from cells lacking TFG3. (A) Immunoblot analysis of immunoprecipitations with anti-SNF6 antibodies and SWI/SNF complex partially purified from *tfg3*Δ extracts. Immune complexes were formed with anti-SNF6 antibodies conjugated to protein A-Sepharose and the peak Mono S fraction as described in Materials and Methods. Immune complexes were washed three times with 1 ml of buffer A containing 500 mM potassium acetate and 0.2% Nonidet P-40 and then boiled for 5 min in 40 μl of SDS loading buffer. To load half of the IP supernatant, the material was precipitated with deoxycholate (0.2%) and trichloroacetic acid (10%). The untreated Mono S fraction (20 μg; approximately 5% SWI/SNF complex; lane 1), half of the precipitated IP supernatant (20 μg, lane 2), and half of the anti-TFG3 immune precipitate (lane 3) were each separated on two SDS–10% acrylamide gels, immunoblotted, and probed with either anti-SNF2, anti-SWI3, or anti-SNF5 antiserum. (B) TFIIF lacking TFG3 does not interact with native SWI/SNF complex. TFIIF lacking TFG3 (TFIIF*) was purified as previously described (18). The peak DEAE-Sephacel fraction of the native SWI/SNF complex (6 μg; 40% SWI/SNF complex) was mixed with 20 μl (100 ng) of near-homogeneous TFIIF*. The sample was rocked at 4°C for 90 min, and 40 μl of 50% protein A-Sepharose beads conjugated to SNF6 antibody was then added. The final potassium acetate concentration in the sample was 160 mM. The sample was rocked for 4 h at 4°C and centrifuged briefly in a centrifuged briefly in a microcentrifuge at 4,000 × *g*, and the supernatant was removed and stored in liquid nitrogen. The pellets were washed extensively and then eluted with two 25-μl aliquots of 5 M urea.

TFG3 protein is not required for the DNA-dependent ATPase activity of SWI/SNF. Highly purified preparations of the SWI/SNF complex contain a potent DNA-dependent ATPase that is associated with the SWI2/SNF2 component (3, 7). To determine whether SWI/SNF complex lacking TFG3 protein retains DNA-dependent ATPase activity, we assayed the supernatant of the anti-SNF6 immunoprecipitation of the SWI/SNF complex lacking TFG3 for this activity. As a control, this peak Mono S fraction was subjected to a mock immunoprecipitation with protein A-Sepharose beads conjugated to preimmune serum. Anti-SNF6 coupled beads were able to immunodeplete the majority of the DNA-dependent ATPase activity; only 25% of the activity remained in the supernatant. In contrast, the mock-treated supernatant exhibited 85% of its original activity. These results suggest that the SWI/SNF complex isolated from *tfg3*Δ cells is associated with a DNA-dependent ATPase activity.

DISCUSSION

Recent studies have demonstrated that many transcriptional activators do not exert their positive effects solely through factors required for basal transcription. Genetic and biochemical studies identified TFIID and SWI/SNF as two large com-

plexes involved in transcriptional activation (11, 33, 43, 60). TFIID, a complex of seven or eight polypeptides in addition to the TATA-binding protein, also participates in both basal and activated transcription (11, 44). Genetic studies with *S. cerevisiae* identified several SWI and SNF polypeptides and suggested that these proteins formed a complex (13, 32, 33, 40, 43, 54). Biochemical studies verified this prediction, confirmed the DNA-dependent ATPase activity of yeast SWI2/SNF2 protein, and revealed the presence of several additional tightly associated polypeptides (3, 7).

In contrast to TFIID and TFIIF, both of which contain at least one component that is required for basal level transcription in vitro, no member of the SWI/SNF complex appears to be required for the basal reaction (3, 18, 25). In addition, many components of TFIID are encoded by essential genes whereas deletions of SWI/SNF complex members confer only slow-growth or conditional phenotypes (18, 32, 43, 45). Genetic and biochemical studies with *S. cerevisiae* and biochemical experiments with mammalian cells all suggest that the primary role of SWI/SNF complex may be to help transcriptional activators bind to sites occluded by chromatin components (6, 7, 23, 30).

This work identifies the 29-kDa member of the yeast SWI/SNF complex as TFG3/TAF30/ANC1. This protein is remarkable in many respects. First, it is tightly associated with TFIIF, TFIID, and the SWI/SNF complex and is thus a member of three complexes known to contribute to transcriptional activation in yeast cells. TFG3 is not a component of TFIIH, TFIIE, or any other characterized yeast basal transcription factor (17a). The phenotypes of *tfg3*Δ strains, however, demonstrate that TFG3 is not essential for many of the tasks associated with these complexes, such as basal-level transcription (TFIID and TFIIF) and nonfermentative growth on various carbon sources (SWI/SNF complex). As further shown here, TFG3 is not required for transcriptional activation by certain activators shown previously to depend strongly on other components of the SWI/SNF complex.

TFIIF and TFIID complexes have been purified to homogeneity from mammalian cells, and almost all of the tightly associated components of these complexes have been cloned and sequenced (14, 18, 21, 22, 28). Neither of these mammalian complexes has been shown to contain a mammalian homolog of yeast TFG3. Despite the lack of a TFG3 homolog as a member of mammalian TFIIF or TFIID, the possibility remains that a TFG3 homolog interacts with these complexes or is present at substoichiometric levels (18).

Our observation that TFG3 occurs in three distinct complexes raises the possibility that it acts as a bridge between these complexes. Since *TFG3* is not an essential gene, these interactions, or the role of TFG3 in these interactions, must not be essential. Furthermore, TFG3 is unlikely to play a crucial structural role in these complexes, because deletions of any of the other components of TFIIF or TFIID confer lethality (18, 45). TFG3 may, however, perform a similar nonessential function in all three complexes, such as assisting in the binding of these complexes to RNA polymerase II or chromatin components. Although we have not been able to demonstrate stable interactions between complexes containing TFG3, many conditions still remain to be tested, including possible interactions with TFIID. Another possibility is that TFG3 masks or protects essential interaction sites on these complexes, possibly regulating the fidelity of interactions between complexes. In strains that lack TFG3, these essential interactions may still take place but may not be properly regulated.

Homologs of *SWI2/SNF2* have been identified in *S. cerevisiae*, *Drosophila melanogaster*, mice, and humans (8, 12, 26, 34, 37, 41, 52). In addition, homologs of *SNF5* have been isolated in both humans and *D. melanogaster*, and both homologs are believed to be associated in a large complex that contains a SWI2/SNF2 homolog (9, 24). A human SWI/SNF complex (hSWI/SNF) has been partially purified, and contains a polypeptide which is a SWI2/SNF2 homolog (24, 30). Sequencing of the other components of hSWI/SNF complex may still reveal a homolog of *TFG3.*

The presence of a SNF5 homolog in hSWI/SNF complex, coupled with the discovery of a SNF5 homolog in flies, suggests that the composition of the complex may be conserved in evolution. Thus, homologs of other yeast SWI/SNF complex members are likely to be found in analogous complexes in other organisms. Here, we report that TFG3 interacts strongly with SNF5 in a far Western analysis, suggesting that TFG3 homologs may interact with SNF5 homologs in higher cells.

Studies on the mammalian homologs of SWI2/SNF2 and SNF5 suggest the involvement of these proteins in diverse and important cellular processes. For example, the human homolog of SNF5, INI1, binds to human immunodeficiency virus type 1 integrase and stimulates its DNA-joining activity 20-fold in vitro (24, 49). In addition, studies on hBrm show a correlation between the expression of hBrm and the ability of the glucocorticoid receptor to activate transcription (37). Consistent with these observations, SWI2/SNF2 is required for glucocorticoid receptor activation in *S. cerevisiae* (60). Furthermore, the ATPase domain of another human SWI2/SNF2 homolog, BRG1, can be swapped for the corresponding domain in SWI2/SNF2 and will enable activation by the glucocorticoid receptor in *S. cerevisiae* (26). Recent studies also suggest that BRG1 binds to the retinoblastoma suppressor protein and may assist this protein in inducing tumor suppressor activity (10).

The SWI/SNF complex may also be involved in leukemogenesis. Reciprocal chromosomal translocations are often associated with acute human leukemias, and many of these translocations result in the fusion of the *HRX* (human trithorax) gene (also called *ALL-1*) to one of several other genes located on other chromosomes (38, 53). *HRX* is significantly similar in two regions to the *Drosophila* transcriptional activator *trithorax* (35). The first region of similarity encodes a zinc finger-containing domain. The other region, which exhibits exceptional similarity (60.5% identical and 82% similar over a 215-amino-acid region), is located at the carboxy terminus of HRX (53). The human protein contains a putative DNA-binding region near the amino terminus composed of "AT hooks", which are believed to be involved in binding to the minor groove of DNA, whereas the *D. melanogaster* protein lacks such a region. Genetic experiments in *D. melanogaster* strongly suggest that the *D. melanogaster* SWI2/SNF2 homolog *brahma* assists certain homeotic activators such as *trithorax* in relieving the repressive effects of chromatin, perhaps through a *D. melanogaster* counterpart of the yeast SWI/SNF complex (51). These results raise the possibility that human HRX protein interacts with a human SWI/SNF complex.

All chromosomal translocations involving HRX result in the production of chimeric proteins, and it has been suggested that these fusion proteins may improperly alter the transcriptional program (38, 53). The HRX fusion proteins retain the amino-terminal DNA-binding region of HRX but do not always retain the carboxy-terminal region. Two of the most common fusions are to the ENL or AF-9 genes, which are strikingly similar to one another. This similarity has led others to suggest that the gene products perform analogous functions in the cell and therefore may perform similar roles in promoting leukemia when fused to HRX protein (38). In addition, both ENL and AF-9 contain nuclear targeting signals, and immunofluores-

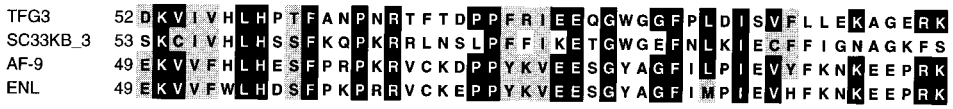

FIG. 5. TFG3 is significantly similar to the human ENL and AF-9 proteins. An alignment of a homologous 50-amino-acid region of TFG3, SC33KB_3, ENL, and AF-9 proteins is shown. The amino acid position of the residue that begins each region is indicated. Residues that are identical among at least three of the proteins are displayed as white letters on a black background, and conserved residues (determined with a PAM-150 matrix) are displayed on a shaded background.

cence experiments demonstrate that ENL resides in the nucleus (38, 46).

Welch and Drubin previously reported that TFG3 is significantly similar to ENL and AF-9 (56). We find that TFG3 is also similar to the predicted polypeptide encoded by the uncharacterized open reading frame SC33KB_3. As calculated by the BLAST program, the probability that the maximum score between the 50 most highly conserved residues of TFG3 and AF-9 would occur at random is 3.9×10^{-10} (Fig. 5). The corresponding probability between TFG3 and ENL is 6.2×10^{-7} (1, 56). The similarity between TFG3 and SC33KB_3 is observed through almost the entire length of these proteins, and the corresponding probability (as calculated by BLAST) is 7.2×10^{-43}. Importantly, the regions of greatest similarity between TFG3 and ENL/AF-9 are the same as those between ENL and AF-9. The homology between *TFG3*, *AF-9*, and *ENL*, together with our observation of TFG3 as a member of the yeast SWI/SNF complex, led us to the proposal that ENL and/or AF-9 protein associates with a human SWI/SNF complex. Whereas activators may ordinarily interact with SWI/SNF components in a regulated fashion, fusion of the HRX activator to ENL or AF-9 may result in persistent interaction with the SWI/SNF complex and thus to uncontrolled access to sites normally occluded by chromatin. The resulting changes in gene expression may play a role in initiating acute leukemias. According to this idea, it is not necessary for the TFG3 homolog to be a positive-acting factor or even a factor required for the activity of SWI/SNF complex; it need only interact with the complex. In addition, the presence of a putative TFG3 homolog in *S. cerevisiae* raises the possibility that this factor is a member of a related complex. Future studies of TFG3 and SC33KB_3 function in *S. cerevisiae* may help us understand how ENL and AF-9 participate in leukemogenesis in humans.

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