Eukaryotic tRNA genes are controlled by proximal and downstream elements that direct transcription by RNA polymerase (pol) III. Transcription factors (TFs) that reside near the initiation site are related in *Saccharomyces cerevisiae* and humans, while those that reside at or downstream of the B box share no recognizable sequence relatedness. Human TFIIICβ is a transcriptional regulator that exhibits no homology to *S. cerevisiae* sequences on its own. We cloned an essential *Schizosaccharomyces pombe* gene that encodes a protein, Sfc6p, with homology to the *S. cerevisiae* TFIIIC subunit, TFC6p, that extends to human TFIIICβ. We also isolated and cloned *S. pombe* homologs of three other TFIIIC subunits, Sfc3p, Sfc4p, and Sfc1p, the latter two of which are conserved from *S. cerevisiae* to humans, while the former shares homology with the *S. cerevisiae* B box-binding homolog only. Sfc6p is a component of a sequence-specific DNA-binding complex that also contains the B box-binding homolog, Sfc3p. Immunoprecipitation of Sfc3p further revealed that Sfc1p, Sfc3p, Sfc4p, and Sfc6p are associated in vivo and that the isolated Sfc3p complex is active for pol III-mediated transcription of a *S. pombe* tRNA gene in vitro. These results establish a link between the downstream pol III TFs in yeast and humans.

RNA polymerase (pol) III is a multisubunit enzyme that is directed to initiate RNA synthesis by transcription factors (TFs) that bind to gene promoter elements. Pol III transcripts comprise a large variety of small nuclear and cytoplasmic RNAs (1). While there is substantial diversity in the promoter structures of pol III-transcribed genes, three major classes are responsible for the synthesis of the more abundant of the cellular pol III transcripts, tRNAs, 5 S rRNA, and U6 snRNA (2). Each of these represents one of three distinct gene classes that utilize a characteristic promoter structure and a specific set of TFs (3). 5 S rRNA genes comprise class I and contain a principal internal promoter that is a binding site for TFIIIA. Class 3 genes utilize upstream TATA elements and, in metazoans, an additional upstream element that binds a distinct multisubunit TF (4). Class 2 is represented by tRNA genes, which are driven by an internal split promoter composed of proximal box A and distal box B elements. In contrast to the diversity of promoter structures, the termination signal for polymerase III transcription is the run of dT residues found at the 3'-ends of pol III-transcribed genes (reviewed in Ref. 5).

The A box usually begins 10–15 base pairs (bp) downstream of the start site of transcription, and the B box is further downstream, the distance depending on the particular tRNA gene. The terminator is the most 3' element, usually found within 20 bp of the B box (6). The multisubunit TF IIC spans the length of tRNA genes, binding to the internal promoter and terminator regions (3). The largest subunit of TFIIIC plays a central role in initiation by recognizing the B box promoter and orienting its associated subunits along the DNA. The TFIIIC subunits that are oriented toward the start site promote TFIIIB binding and therefore assist in directing accurate initiation by pol III (7–11). Much less is known about the functions of the downstream TFIIIC subunits, TFC6p in *Saccharomyces cerevisiae* and TFIIICβ in humans, within their transcription complexes (12–15) (see below).

The TFIIIB subunits, TATA-binding protein (TBP), and TFIIH-related factor (Brf), have been conserved from *S. cerevisiae* to humans, as have the two TFIIIC subunits that localize near the A box and several subunits of pol III itself (10, 16–21). By contrast to these initiation factors, the downstream TFIIIC subunits in these organisms had revealed no recognizable sequence relatedness. For example, hTFIIIC220, the human polypeptide that binds the B box, bears no homology to the *S. cerevisiae* B box-binding factor, TFC3p, or to anything in the *S. cerevisiae* sequence database (22, 23). Some *S. cerevisiae* TFIIIC subunits, TFC7p and TFC8p, as well as the downstream protein, TFC6p, were reported to have no human homologs (13, 25–27).

Another example of divergence is provided by hTFIIICβ (also known as hTFIIIC110 (10)), a pol III regulatory factor that activates transcription during cellular proliferation and in response to adenovirus E1A (15). A critical feature that distinguishes TFIIIC complexes that bind the B box but are inactive for transcription from those that bind and are active is the presence of hTFIIICβ in the latter (15, 28). Although hTFIIICβ contains intrinsic histone acetyltransferase activity that relieves chromatin-mediated repression, this is presumably not its sole activity, since it is also required for transcription of...
naked templates in a highly purified system, (15, 29). Examination of the interaction of hTFIIICβ with hTFIIIC220 suggested that it is oriented downstream of the B box, toward the 3′-region of the transcription complex (14). Thus, the mechanism by which hTFIIICβ activates transcription has remained an intriguing puzzle with no clues from the S. cerevisiae system (10, 15).

Our laboratory has begun to examine pol III transcription in the fission yeast Schizosaccharomyces pombe. For the present report, we identified a sequence in S. pombe, designated sfc6 (S. pombe TFC6) that exhibits highly significant homology to both TFC6p and hTFIIICβ. This led to the identification, cloning, isolation, and expression of four subunits of a S. pombe TFIIIC complex. Specific promoter binding and transcription activity demonstrate that Sfc6p is an integral component of an active S. pombe TFIIIC. Since homology between human hTFIIICβ and yeast TFC6p went unrecognized previously and is not apparent without the Sfc6p sequence, this study demonstrates that the S. pombe system is uniquely valuable in extending the relatedness of the pol III systems of yeast and higher eukaryotes while simultaneously establishing a TFIIIC-dependent transcription system in this alternative model organism.

MATERIALS AND METHODS

Growth of S. pombe—The strains used for this study are described in Table 1. Yeast extract plus supplements or Edinburgh minimal medium plus gln-agar was used for routine culture or selection of transformants (30). Edinburgh minimal medium plus 5 μg/ml phloxin B was used to differentiate diploid from haploid cells (30). Edinburgh minimal medium plus 5 μg/ml phloxin B was used to differentiate diploid from haploid cells (30).

| Strain | Genotype | Purpose | Source |
|--------|----------|---------|--------|
| yHL972 | h−       | Isolation of sfc6 gene | H. Levin |
| yHL975 | h+       | Mating type test strain | H. Levin |
| yHL1101| h+, ade6-M210, ura4-D18, leu1–32 | ade6 allele test strain | H. Levin |
| yHL1102| h+, ade6-M216, ura4-D18, leu1–32 | ade6 allele test strain | H. Levin |
| SP1190 | h−, ade6-M704, leu1–32, ura4− | To test FH-Sfc6 expression | ATCC |
| yHL8818| h+/h−, his3-D1, leu1–32, ura4-D18, ade6-M210/ade6-M216 | sfc6 gene disruption | H. Levin |
| yYH8048| h+/h−, his3-D1, leu1–32, ura4-D18, ade6-M210/M216, sfc6+/Δsfc6::his3+ | Isolation of Δsfc6::his3+ | This Laboratory |
| yYH8238| h−, his3-D1, leu1–32, ura4-D18, ade6-M216 | Affinity purification | This Laboratory |
| yHL6382 | h−, his3-D1, leu1–32, ura4-D18, ade6-M216 | Affinity purification of TFIIIC | This Laboratory |
| yYH2230| h−, ade6-M216, his3-D1, leu1–32, ura4-D18, Δsfc3::[FH-Sfc3-His3+] | | |

Plasmids for Bacterial Expression—A sense primer TFC1sen2 (5′-CATATGCCATATGCTAATGATGGTATGTA-3′), and a antisense primer, TFC1ant1 (5′-GCTAGCTAATGATGGTATGTA-3′) was used to generate plasmids for bacterial expression. The plasmids were constructed in pET28a (Novagen, Madison, WI).

Table I. Yeast extract plus supplements or Edinburgh minimal medium containing the 5′-flanking region upstream of the sfc3-Δ promoter was amplified by PCR and cloned into the KpnI–SalI sites of pAF-Sfc3, resulting in pAF-Sfc3in, which was used to construct the integrant S. pombe strain yYH2230.

Plasmids for Yeast Expression—To construct plasmids for yeast expression, the primers were “N terminus 1” (5′-GGGATCCGTCGACAGGGCCCATGGCGCTA-3′) and an antisense primer, TFC-1 ant1 (5′-GCTAGCTAATGATGGTATGTA-3′) was used to construct plasmids for yeast expression. The plasmids were constructed in pET28a (Novagen, Madison, WI).
The prototrophic hin3 transformants were selected on the appropriate media. Southern blotting was performed by digesting 5 µg of genomic DNAs with AgeI and Ncol. After fractionation on a 1% agarose gel, the DNA was denatured, neutralized, and transferred to a nylon membrane (GeneScreen Plus; NEN Life Science Products). The membrane was prehybridized in buffer containing NF (44). Spots of DNA were revealed using 0.5% SDS, 0.1 mg/ml denatured herring sperm DNA, and 50% formamide at 42°C for 2 h. A 4.0-kb fragment containing the open reading frame of sfc3 was labeled by random primer extension to 4 × 10⁶ dpm/ml (Lofstrand Laboratories) and added to a final concentration of 10³ dpm/ml. After incubation at 42°C for 16 h, the membrane was washed twice in 2× SSC and then incubated with 1 ml of buffer containing 20 mM HEPES, pH 7.9, 20% glycerol, 0.2 mM EDTA, 2 mM dithiothreitol, 0.1 mM PMSF, 150 mM NaCl, 0.1% Nonidet P-40. The bound material was eluted with 20 µl of 0.2 mg/ml FLAG in the same buffer.

RESULTS

Identification of a S. pombe Protein, Sfc6p, with Homology to S. cerevisiae TFIIIC and hTFIIICβ—A search of the S. pombe sequence data base using S. cerevisiae TF6p as a query identified a predicted protein with a mass of 66.2 kDa encoded by four exons on chromosome II that is also homologous to hTFIIICβ (45). We designated the gene for this protein sfc6. A cDNA in which the four predicted exons had apparently been spliced was isolated from a S. pombe cDNA library and sequenced (not shown, but see Fig. 1). This sequence encodes a protein of 562 amino acids with 25% identity (41% similarity) to TFIIIC and 19% identity (33% similarity) to hTFIIICβ (Fig. 1). After two iterations, PSI-BLAST included only three proteins, S6fp, TFC6, and hTFC6, in the set of highly significant homologs, producing alignment scores above 400 and e values of 10⁻¹⁴² and 10⁻¹²³ for TFC6p and hTFIIICβ, respectively (45). An acidic region previously noted in TFC6 is conserved in Sfc6p (residues 16–42) and to a significant degree in the corresponding region of hTFIIICβ (residues 40–74) (13, 15). Sfc6p, TFC6p, and hTFC6p each contain WD-40 repeats in their C-terminal regions, as predicted by Motif 3 and reported for hTFIIICβ (15). Sfc6p and TFC6p exhibit HMG-I and HMG-Y (A + T hook) motifs at their N termini, while this motif is predicted at positions 178–190 and again at 270–282 for hTFIIICβ.

Sfc6p Is Encoded by an Essential Gene Whose Product Is a Component of a tRNA Gene Promoter Recognition Complex in S. pombe—We deleted the protein-coding region of sfc6 from one allele of a diploid strain, replacing it with the hin33 gene, and confirmed the deletion by PCR (not shown). Of multiple asc di dissected, none yielded more than two viable spores, all of which failed to grow on media lacking histidine, indicating that sfc6 is essential for viability and/or germination (not shown). Transformation of the sfc6::hins3 diploid with pREP90X-FH-S6fp, followed by sporulation, led to histidine and leucine prototrophs that expressed FH-S6fp (not shown, but see below). The inability to recover haploids that were histidine prototrophs after transformation with pREP4X-F-S6fp and counterselection with 5-fluoroorotic acid established that sfc6 is essential for viability (not shown).

Purification of FH-S6fp from S. pombe revealed an associated DNA binding activity that recognized the promoter of a tRNA gene (Fig. 2). The 85-bp tDNA probe used for this experiment contains no sequences upstream of where transcription would start and should therefore be specific for TFIIIC (see “Materials and Methods”). Sequential anti-FLAG and nickel-mediated affinity purification of extract from cells expressing FH-S6fp yielded significant tDNA binding activity (Fig. 2, A and B, lanes 1, 2), while the subsequent purification scheme yielded no activity from extract of control cells that do not express FH-
Sfc6p (Fig. 2, A and B, lanes 1). The sequential affinity purification scheme led to a large increase in the EMSA specific activity, since unpurified extract exhibited undetectable activity in this assay (not shown). SDS-PAGE followed by silver staining revealed several bands, the most abundant of which was identified as Sfc6p, which was overexpressed relative to endogenous levels of Sfc6p, while another abundant species was identified as S. pombe heat shock 70 protein (not shown).

We concluded from this that overexpression of Sfc6p led to association with the heat shock 70 chaperone. Attempts to purify the complex using lower levels of Sfc6p expression with the purpose of identifying stoichiometrically relevant, specific components are under way. A specific activity of TFIIIC, its ability to recognize a tRNA gene promoter in the EMSA, is examined below.

Sequence-specific binding and demonstration that Sfc6p is present in the tDNA-binding complex are shown in Fig. 2C. While the unlabeled tDNA competed for binding (Fig. 2C, lane 2), an unlabeled probe containing nucleotide substitutions in the A and B boxes competed less efficiently (lane 3). We demonstrated that FH-Sfc6p was a component of the DNA binding activity by examining the effect of anti-FLAG Ab in EMSA reactions (Fig. 2C, lanes 5, 7, and 8). This Ab caused a supershift in the mobility of the bound probe (lane 5), while control Ab exhibited a nonspecific inhibitory effect but did not cause a supershift (lane 6). Moreover, the supershift caused by anti-FLAG could be competed by FLAG peptide (lane 7) but not by an unrelated peptide at the same concentration (lane 8). Unlike Sfc6p purified from S. pombe, recombinant Sfc6p affinity-purified from bacteria exhibited no significant binding to the probe even at relatively high concentration (data not shown), similar to what has been reported for recombinant TFC6p (13).

Isolation of Sfc4p and Sfc1p, S. pombe Homologs of Conserved TFIIIC Subunits—PSI-BLAST (45) identified S. pombe homologs of two proximally oriented TFIIIC subunits; the first is designated TFC4p or PCF1p in S. cerevisiae and hTFIIIC102 in humans, and the second is designated TFC1p in S. cerevisiae and hTFIIIC63 in humans (10, 47–50). The corresponding pre-
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FIG. 2. Fission yeast Sfc6p is an integral component of a tRNA promoter-binding activity. A, EMSA using a double-stranded tDNA probe. Binding reactions contained 2 μl of material isolated by sequential anti-FLAG and nickel-agarose-mediated affinity purification of extract prepared from control cells (lane 1) or from cells that express FH-Sfc6p (lane 2). B, immunoblot analysis of the material in A, isolated from control cells (lane 1) or cells expressing FH-Sfc6p (lane 2). The positions of size markers that were coelectrophoresed are indicated on the left in kDa. C, the affinity-purified FH-Sfc6p-associated tDNA-binding activity was examined by EMSA as in A and also in the presence of additional components as indicated above the lanes and described under “Materials and Methods.” Specific and mutated DNA probes are indicated. Directed S. pombe proteins were designated Sfc1p and Sfc4p and have predicted masses of 52.7 and 116.4 kDa respectively. Sfc1p exhibits significant homology to TFC1p and hTFIIIC63, with e values of $10^{-128}$ and $10^{-67}$, respectively, after two iterations (45). Sfc4p exhibits significant homology to TFC4p and hTFIIIC102 with e values of $10^{-151}$ and $10^{-152}$, respectively, after two iterations (45). The genomic sequence for Sfc1p was interrupted by a single intron, while Sfc4p was predicted from a single open reading frame. The coding sequences of these were cloned from cDNA and genomic DNA, respectively, and expressed in recombinant form, confirming that each generated a protein of the expected size (see below).

Isolation of Sfc3p, a B Box-binding Homolog, and an Associated TFIIIC Complex from S. pombe Cells—Although the Sfc6p complex exhibited sequence-specific DNA binding to a tRNA gene, recovery was poor, perhaps due to limited accessibility of the N-terminal Sfc6p epitope tag as reported for TFC6p (13). Therefore, when the S. pombe TFIIIC complex was puriﬁed from S. pombe, the S. cerevisiae B-box-binding protein, became available, we examined its potential involvement in the putative S. pombe TFIIIC complex. The TFC3p sequence identiﬁed a single predicted protein sequence in the S. pombe data base that exhibited 21% identity and 39% similarity that extended over 1339 amino acids (not shown). We designated this predicted protein Sfc3p. In this case, no homology between Sfc3p and hTFIIIC220, the human B box binding subunit, could be demonstrated upon reiterations using PSI-BLAST (not shown). The predicted Sfc3p, encoded by a single open reading frame, was cloned with FH epitope tags on its N terminus and expressed from a plasmid in S. pombe. This generated a protein of the expected size (154 kDa), conﬁrming its coding capacity in vivo (not shown, but see below).

A S. pombe strain containing an FH version of Sfc3p was created by homologous recombination. Southern blotting identiﬁed sfc3” as a single copy gene in wild type S. pombe cells and also conﬁrmed the genomic structure of the FH-sfc3 integrant, demonstrating that FH-sfc3 was the only copy of sfc3” in this strain (data not shown). Extracts prepared from the FH-Sfc3 strain, a control strain grown under identical selective conditions, and a wild type strain grown under nonselective conditions were incubated with M2-agarose. After incubation, the supernatants were collected as the ﬂow-through, the agarose was washed ﬁve times with buffer containing 250 mM NaCl, and the bound material was eluted. The input (I), ﬂow-through (F), and eluate (E) of the M2-agarose from the three extracts were fractionated by SDS-PAGE and analyzed by immunoblotting using antisera to ﬁve different proteins. A, anti-Sfc4p raised against an amino-terminal peptide. B, anti-Sfc6p raised against full-length protein. C, anti-Sfc1p raised against full-length protein. D, anti-TBP raised against full-length protein. E, anti-Sfc3p raised against a 28-kDa polypeptide comprising 243 amino acids at the C terminus. Size markers were coelectrophoresed on each gel and are indicated on the left in kDa. The positive control proteins were from pREP90X-FH-Sfc4 expressed in S. pombe, pREP4X-F-Sfc6 expressed in S. pombe, pREP90X-FH-Sfc1 expressed in S. pombe, pET28a-HspTBP expressed in bacteria, and pREP4X-F-Sfc3 expressed in S. pombe.
ent in the eluate of the FH-Sfc3 strain (lane 6) but not in the eluate of the control strains (lanes 3 and 9). SDS-PAGE followed by silver staining revealed several bands, the mobilities of the most abundant of which corresponded to Sfc4p, Sfc1p, and Sfc6p, as well as other bands of unknown identity, in addition to several bands of lower apparent stoichiometry (not shown). The data demonstrated that all four of the yeast TFIIIC homologs that were identified and characterized here are associated in vivo. By comparison, FH-Sfc6p-containing strains constructed by the same approach as FH-Sfc3p, yielded substantially less FH-Sfc6p-associated Sfc4p and Sfc1p than the FH-Sfc3p complex, again perhaps due to limited accessibility of the N-terminal epitope tag as reported for TFC6p (not shown; see Ref. 13). Therefore, we focus on the Sfc3p complex for the remainder of this report.

In Vitro tRNA Gene Transcription in a S. pombe Extract—The availability of a newly described extract of S. pombe that is active for tRNA transcription (44) provided an opportunity to examine the potential involvement of the Sfc3p complex in pol III transcription. The tRNASer\(^{\mathrm{UGA}}\)M gene, modified from the S. pombe sup3-e (43), was used for this purpose. In one version of this template, designated tRNA\(_{\mathrm{UGA}}\)Ser\(_{\mathrm{UGA}}\)M-T7, the tRNA sequence is followed by a functional pol III terminator consisting of seven T residues to produce a primary transcript of 112 nucleotides, similar to other eukaryotic precursor tRNAs. In addition to a major band that corresponds to a primary transcript, this template also yielded lower bands that are probably the result of posttranscriptional processing of the nascent precursor tRNA, which is known to occur in comparable extract systems (43).

Another version of the template, designated tRNA\(_{\mathrm{UGA}}\)Ser\(_{\mathrm{UGA}}\)M-3T, does not contain a functional terminator following the tRNA sequence and produces read-through transcription to a downstream terminator of eight T residues, to generate a more distinctive transcript of 210 nucleotides (44).

Fig. 4A shows the products of transcription reactions that contained no added template (lane 1), tRNA\(_{\mathrm{UGA}}\)Ser\(_{\mathrm{UGA}}\)M-3T (lane 2), and tRNA\(_{\mathrm{UGA}}\)Ser\(_{\mathrm{UGA}}\)M-T7 (lane 3). The sizes of the template-dependent transcripts were as expected for tRNA promoter-dependent transcription. Since these templates differ only by the deliberate absence or presence of a functional terminator following the tRNA sequence, the difference in size is a strong indication that both directed comparable start site selection by pol III.

The different sizes of these in vitro transcription products would then indicate that initiation occurs at or very close to the 5′-initiation site previously mapped for the tRNA\(_{\mathrm{UGA}}\)M gene from which tRNA\(_{\mathrm{UGA}}\)Ser\(_{\mathrm{UGA}}\)M was derived (51, 52). Lanes 4 and 5 show the products of reactions that differed from those in lanes 2 and 3 only by the lack of UTP in the latter. The filled arrow indicates the major transcript of the tRNA\(_{\mathrm{UGA}}\)Ser\(_{\mathrm{UGA}}\)M-3T gene, while the open arrowhead indicates the major transcript of the tRNA\(_{\mathrm{UGA}}\)Ser\(_{\mathrm{UGA}}\)M-T7 gene (see “Results”). The band indicated as IC appears not to be a product of pol III transcription, since it is resistant to high concentrations of tagetitoxin and α-amanitin (data not shown), but it serves as an internal control. B, extract from cells expressing FH-Sfc3p was used without depletion (lane 1) or incubated with protein A-agarose (pA, lane 2) or M2-agarose (lanes 3–5) prior to use in the in vitro transcription assay. The extract samples were then supplemented by the addition of buffer alone (lanes 1–3), a control eluate (c, lane 4), or eluate from M2-agarose (lane 5). The arrow on the right indicates the position of the pol III-dependent, promoter-mediated, tRNA gene-derived transcript (see “Materials and Methods”). A recovery marker (-RM) and internal control (-IC) are indicated on the right.

Transcription of tRNA\(_{\mathrm{UGA}}\)Ser\(_{\mathrm{UGA}}\)M-3T is reflected by the 210-nucleotide transcript in lane 1 (arrow). Note that a 32P-labeled DNA recovery marker (indicated by -RM) was added to the transcription reactions in Fig. 4B. While a mock depletion with control protein A-agarose (pA) led to minimal inhibition of transcription (lane 2), depletion with M2-agarose more significantly inhibited transcription activity but did not deplete the internal control marker (IC, lane 3). This indicated that a positive activity required for tRNA transcription was specifically depleted from the extract by M2-agarose. Moreover, while the addition of the control eluate did not restore activity to the M2-depleted extract (lane 4), the eluate from M2-agarose restored the activity (lane 5). The recovery marker (-RM) as well as the internal control (-IC), provided further evidence that the M2-agarose-mediated depletion was specific and reversible. The ability of the M2-agarose eluate to restore transcription represents its activity. The data indicate that the TFIIIC complex that was isolated from S. pombe is active.

DISCUSSION

TFIIIC is a multisubunit transcription factor that has been well characterized in S. cerevisiae and human in vitro transcription systems that is required for the synthesis of tRNAs (8, 10, 46, 54). We isolated from S. pombe the coding sequences as well as the proteins themselves, representing four subunits of TFIIIC. Homologs of two of these had been known (TFC1/ hTFIIIC63 and TFC4/hTFIIIC102) (10), while characterization of the other two as reported here extends our understanding of the pol III systems in yeast and humans. Sequence relatedness alone does not indicate that homologous proteins serve orthologous functions. Therefore, it was imperative that Sfc6p be characterized functionally.

A specific conclusion that can be made from this work is that
The Sf6p is homologous to S. cerevisiae TFC6 and hTFIIICβ, neither of which reveals sequence relatedness to the other on its own. Genetic and physical data indicate that the N-terminal third of TFC3p interacts with TFC6p and that the latter is the most downstream of the DNA-binding TFIIC subunits (12, 13). Similarly, the N-terminal fragment of hTFIIIC220 appears to interact with hTFIIICβ, with the latter oriented downstream (14). Evidence from sequence-specific promoter binding, association with conserved subunits of TFIIC, and transcription factor activity leave no doubt that Sf6p is a bona fide component of S. pombe TFIIC. Furthermore, by revealing that Sf6p is an integral subunit of TFIIC that is related to hTFIIICβ, Sf6p served a unique and important role in extending the relatedness of the pol III systems of yeast and humans. This is significant because of a disparity that contrasted the evolutionary conservation of the upstream pol III TFs with the lack of conservation of the downstream factors, including the core subunit, the B box-binding protein, and hTFIIICβ (10). These data further suggest that the Sf6p-related factors were derived from a common ancestral sequence that has diverged substantially in humans and S. cerevisiae, to the point where TFC6p and hTFIIICβ show no sequence homology when compared only with each other.

The C-terminal regions of TFC6p, Sf6p, and hTFIIICβ contain WD-40 repeats, which are of potential importance for protein-protein interactions (see Ref. 15). These sequences also share homology in their N-terminal regions, and each exhibits predicted HMG-I and HMG-Y (A + T hook) motifs. This motif may provide a clue to the mechanism of DNA binding by these factors, since TFC6 can be cross-linked to the T-rich termination regions of tRNA and 5 S rRNA genes (3, 12).

A query using hTFIIIC90 returns a predicted S. pombe protein, which, upon reiterations to convergence with PSI-BLAST (45), reveals an e value of \(6 \times 10^{-64}\) for the S. pombe protein but no significant homology to any S. cerevisiae sequence (not shown). Thus, it appears that while our analysis extends the relatedness of the yeast and human pol III systems, it also emphasizes divergence. As another example, S. pombe Sf6p shows significant homology to TFC3p, the B box-binding subunit, while no sequence homology to the human B box-binding subunit, hTFIIIC220, could be discerned (23). Moreover, although TFC6 from yeast and humans have been shown to function in relieving chromatin-mediated repression, this has not been clear that these operate in a similar manner to achieve this. While three of the human TFIIC subunits, TFIICβ, TFIIC220, and TFIIC90, exhibit histone acetyltransferase activity (11, 29), this activity is not readily apparent for S. cerevisiae TFIIC (24). Consistent with the higher relatedness of Sf6p to TFC6p than to TFIICβ, Sf6p exhibits no histone acetyltransferase activity, either in native form expressed in S. pombe or after purification from bacteria, when assayed using highly sensitive conditions. It is interesting in this regard that the three human TFIIC subunits that are endowed with histone acetyltransferase activity, hTFIIIC90, hTFIIICβ, and hTFIIIC220, exhibit the least homology with S. cerevisiae TFIIC subunits. Thus, it would seem as if yeast and human TFIIC both function to relieve chromatin-mediated repression but do so by different mechanisms.

A line of evidence indicates that TFIIC is increased in response to growth factors and adenovirus; elucidation of the importance of hTFIIICβ in these processes revealed this factor as a central regulatory component of pol III transcription in human cells (reviewed in Ref. 15). However, the inability to identify a hTFIIICβ homolog suggested that our understanding of this key factor might not benefit from what is known about yeast TFIIC or the advantages of a genetically tractable system. The availability of a strain of S. pombe in which the homologous essential gene has been functionally characterized and shown to comprise an orthologous subunit of TFIIC should facilitate investigations of this factor. Although we were unable to rescue a strain carrying the null allele of sf6p with hTFIIICβ or TFC6 (not shown), domain swapping may be helpful in the future.

The S. pombe pol III system described here should be a useful adjunct to the other pol III model systems being studied. In this regard, it should be emphasized that no homology between TFC6p and hTFIIICβ could be identified even when comparing these proteins directly using the Blast 2 sequences program, while their homology became readily obvious after the S. pombe Sf6p sequence became available.

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