**Interaction between Yeast RNA Polymerase III and Transcription Factor TFIIIC via ABC10α and γ131 Subunits**

(Received for publication, July 28, 1999, and in revised form, September 1, 1999)

Hélène Dumay‡, Liudmilla Rubbiš, André Sentenac, and Christian Mareck¶

*From the Service de Biochimie et de Génétique Moléculaire, Bâtiment 142, CEA/Saclay, Gif-sur-Yvette, F-91919 Cedex, France*

Yeast TFIIIC mediates transcription of class III genes by promoting the assembly of a stable TFIIIB-DNA complex that is sufficient for RNA polymerase III recruitment and function. Unexpectedly, we found an interaction in vivo and in vitro between the TFIIIB-recruiting subunit of TFIIIC, γ131, and ABC10α, a small essential subunit common to the three forms of nuclear RNA polymerases. This interaction was mapped to the C-terminal region of ABC10α. A thermosensitive mutation in the C terminus region of ABC10α (rpm1-30) was found to be selectively suppressed by overexpression of a mutant form of γ131 (γ131-ATPR2) that lacks the second TPR repeat. Remarkably, the rpm1-30 mutation weakened the ABC10α-γ131 interaction, and the suppressive mutation, γ131-ATPR2 increased the interaction between the two proteins in the two-hybrid assay. These results point to the potential importance of a functional contact between TFIIIC and RNA polymerase III.

In eukaryotic cells, RNA polymerase (Pol)1 III is responsible for the transcription of genes encoding tRNAs, 5 S RNA, U6 RNA, and a number of small RNA species. In tRNA genes (tDNA), the internal promoter elements, the A and B blocks, are recognized by TFIIIC. DNA-bound TFIIIC then directs the assembly of TFIIIB that, in turn, is sufficient to recruit RNA polymerase III for multiple transcription cycles. The transcription of all yeast class III genes is a variation of this scheme (1). TFIIIC and TFIIIB are multiprotein complexes. Yeast Saccharomyces cerevisiae TFIIIC, also called γ, is a large transcription factor (about 550–600 kDa) that comprises six polypeptides, γ138, γ131, γ95, γ91, γ60, and γ55 (2–4), that have been characterized by gene cloning and mutagenesis (5–11). Much insight on TFIIIC-tDNA complex has come from the localization of the various subunits along the tDNA by site-specific protein-DNA cross-linking experiments (3, 12). The most 3′ subunit, γ91 (12), participates in DNA binding with γ138 (10), which is located within and around the B block (3), whereas γ95 and γ55 are accessible to DNA cross-linking within the A block region (3). Finally, the second largest subunit of TFIIIC, γ131, is located the most upstream within the TFIIIB binding region and also extends downstream between the A and B blocks (3). Remarkably, this subunit contains 11 tetratricopeptide repeats (TPR) (8) known to mediate protein-protein interactions (13). γ131 was shown to interact with two components of TFIIIB, TFIIIB70/BRF1 (14, 15) and TFIIIB90B′ (16), and the TFIIIB70/BRF1-interacting domain of γ131 was found to lie in the N-terminal region that includes the first TPR unit (15). Recently, another subunit of TFIIIC, γ60, was found to participate in TFIIIB recruitment via its interaction with TBP (17).

*S. cerevisiae* RNA polymerase (Pol) III is a multisubunit complex comprising 17 polypeptides ranging from 162 to 7.7 kDa (18), five of which, ABC27, ABC23, ABC14.5, ABC10α, and ABC10β, are shared with Pol I and II. A labile triad of subunits, C34, C31, and C82, has been implicated in the recruitment of Pol III and in transcription initiation (19). A mutation in C31 subunit was found to specifically affect transcription initiation but not the catalytic properties of the enzyme (20). C34 was found to be localized the furthest upstream on tDNA in initiation complexes (21, 22), and analysis of mutant Pol III showed that mutations in C34 that decreased its interaction with TFIIIB70/BRF1 affected Pol III recruitment and open complex formation (23). This triad of subunits has its counterpart in human Pol III. These subunits form a subcomplex that is required for transcription initiation (24). One (hRPC39) of these subunits, homologous to γC34, interacts physically with two components of hTFIIIB (hTBP and hTFIIIB90). More recently, a new essential subunit of yeast Pol III, C17, was also found to interact with C31 and TFIIIB70/BRF1 thus adding a new linkage to the TFIIIB-Pol III connection.2 These findings suggest that the recruitment, correct positioning, and activation of Pol III is mediated by multiple contacts between the enzyme and TFIIIB components.

In this work we report genetic and biochemical evidence in favor of a direct contact between yeast Pol III and the assembly factor TFIIIC, namely between the common subunit ABC10α and the TFIIIB-assembling subunit of TFIIIC, γ131. Supporting initial two-hybrid experiments, recombinant ABC10α was found to interact in vitro with γ131. A thermosensitive mutation in the conserved C-terminal region of ABC10α, that weakens this interaction, can be rescued by overexpression of a variant form of γ131. These data suggest the existence of functional interactions between TFIIIC and Pol III.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains, Media, and Genetic Methods**—The yeast strains used in this study were constructed by genetic techniques based on transformation of lithium acetate-treated cells with standard media and growth conditions (25). Yeast strains are as follows: YLR-01 (Mat a ura3-52

2 M. L. Ferri, G. Peyroche, M. Siaut, O. Lefebvre, C. Carles, C. Conesa, and A. Sentenac, submitted for publication.
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trp1 his3-220 vac8 ade2 ade2Δ3 rpc10-16 HIS3 + pGenR-RP10C (26); YLR-06 (Mat a ura3-52 trp1 his3-220 vac8 ade2 ade2Δ3 rpc10-16 HIS3 + pGenR-RP10C (26); YLR-03 (Mat a ura3-52 trp1 his3-220 vac8 ade2 ade2Δ3 rpc10-16 HIS3 + pGenR-RP10C (11)); MW670 (Mat a ura3-52 trp1 his3-220 vac8 ade2 ade2Δ3 rpc10-16 leu1-11 leu1-30 rpc10-16Δ1 H/SIS + pCEN116-120 TRP1 CEN4 pRP10C (12)); MW2092 (Mat a ura3-52 trp1 his3-220 vac8 ade2 ade2Δ3 rpc10-16 leu1-11 leu1-30 rpc10-16Δ1 H/SIS + pCEN116-120 TRP1 CEN4 pRP10C (20)); SC91 (Mat a ura3-52 trp1 his3-220 vac8 ade2 ade2Δ3 rpc10-16 leu1-11 leu1-30 rpc10-16Δ1 H/SIS + pTR1 pRP325/424 (24)); D132-1D (Mat a ura3-52 trp1 his3-220 vac8 ade2 ade2Δ3 rpc10-16 leu1-11 leu1-30 rpc10-16Δ1 H/SIS + pCEN116CAR4R (26) (see Fig. 3A).

Two-hybrid Assays—Two-hybrid system vectors carrying RPC10 mutant alleles were constructed by cloning BamHI-BeI fragments of pGen-RP10C derivatives (rpc10-14, rpc10-15, rpc10-16, rpc10-30, rpc10-24, and rpc10-11) into pAS-JR (15) for fusion with GAL4 DNA-binding domain (residues 1–147). Correct in frame fusion and similar expression level of fusion proteins were confirmed by sequencing and immuno blotting analyses. Two-hybrid systems thus constituted were used to transform YM526 yeast strain. Independent transformants for each combination of plasmids were grown as patches for 2 days at 30 °C on selective solid medium containing 2% raffinose as carbon source. β-galactosidase activity was revealed by overlaying cells with 10 ml of 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal) agar and incubating plates for 24 h at 37 °C or assayed as described previously (19). The interaction between TFIIB70 and RPC11 was as used as a reference (15).

Preparation of Recombinant ABC10α Protein—pRSETthio/RPC10α (kindly provided by J.-M. Buhrer) was generated by subcloning the entire RPC10α coding sequence (obtained by polymerase chain reaction from genomic DNA) in the T7 polymerase expression vector pSETA (Invitrogen) at a BamHI site. This construct produced a ABC10α-thioredoxin fusion protein, tagged with six histidines and T7-Tag TM at the N terminus of ABC10α. Formation of inclusion bodies in the Escherichia coli cytoplasm was prevented by the thioredoxin moiety. E. coli strain BL21 (DE3) (pLYsE) was transformed with pRSETthio/RPC10α, and cultures were grown at 37 °C for 3 h. The induced cultures were harvested by centrifugation and resuspended in binding buffer (50 mM imidazole, 500 mM NaCl, 20 mM Tris-HCl, pH 7.9, protease inhibitors (Roche Molecular Biochemicals) and lysed by heat shock and treatment with lysozyme (0.1 mg/ml final). The lysate was centrifuged, and the protein extract was added to the isopropylthiogalactoside beads equilibrated in the binding buffer. After 1 h at 4 °C, the flow-through fraction was removed, and the resin was washed with binding buffer containing 60 mM imidazole. Bound proteins were eluted stepwise with elution buffer (1 M nitrilotriacetic acid-agarose as specified by the manufacturer (Qiagen) with minor modifications as follows. Bacteria were harvested by centrifugation and resuspended in binding buffer (50 mM imidazole, 500 mM NaCl, 20 mM Tris-HCl, pH 7.9). Samples of eluates were analyzed by Western blotting with anti-T7-Tag TM antibodies (Novagen).

Interaction of ABC10α with 5S-S-131 and 5S-S-131-ΔTRPR2—The BamHI-BamHI fragment of plasmid pASr131 (15) was cloned into the pET28c (Novagen) plasmid to produce the wild-type 5S-S-131 α protein. The BamHI-BamHI fragment of pACTΔTRPR2 (15) was cloned into pET28a (Novagen) to produce the mutant 5S-S-131-ΔTRPR2 protein (lacking amino acids 162–195) (15). These expression plasmids, pET131 and pETΔTRPR2, were linearized using Asel and XhoI, respectively. The genes were transcribed and translated in vitro with T7-Coupled Wheat Germ Extract Systems (Promega) in the presence of [35S]methionine. Expression of 5S-S-131 (150,000 cpm/ml) and 5S-S-131-ΔTRPR2 (100,000 cpm/ml) was verified by SDS-PAGE. Partially purified rABC10α-thioredoxin fusion, purified recombinant thioredoxin (Promega), and a control protein extract from E. coli were subjected to SDS-PAGE and blotted onto nitrocellulose for far-Western analysis (29). The filter-bound proteins were subjected to denaturation/reannotation treatment according to the method of Papavassiliou and Bohmann (30). The interaction of the binding of 5S-S-131, the 5S-labeled background had to be reduced by addition of 5% low fat milk to the probe. This process was not necessary when probing with 5S-S-131-ΔTRPR2 due to a stronger interaction of the mutant protein with ABC10α. Full size rABC10α was revealed by anti-T7-Tag TM antibodies. Immune complexes were visualized using the ECL chemiluminescence kit (Amer sham Pharmacia Biotech), and the bound 5S-labeled polypeptides were detected by autoradiography.

Multi-copy Suppression Assays—The plasmids used for multi-copy suppression experiments were constructed as follows: the SalI-XmaI fragments from pCK14 (8) and pNC14 (15) were cloned into pFL44L to obtain multi-copies plasmids bearing TFC4 wild-type gene (pFL131) and mutant gene TFC4-ΔTRPR2 (pFL44TRPR2) overexpressing r131 and r131-ΔTRPR2 proteins, respectively. pFL44-RP10C has been previously described (31).

Δ131 Mutant Searches—Sequence data for Candida albicans was obtained from the Stanford DNA Sequencing and Technology Center website. Sequencing of C. albicans was accomplished with the support of the NIDR and the Burroughs Wellcome Fund. The sequence of the ABC10α C. albicans ortholog was identified in the unpublished sequence con4–2986 using the NCBI Blast server and the S. cerevisiae sequence as entry. The sequence of ABC10α ortholog in Arabidopsis thaliana has been disclosed using TblastN 2.0 (32) run on the NCBI Blast server and non-redundant DNA data base with the human ABC10α sequence as entry. This protein sequence has been tentatively reconstituted from genomic data (2 introns are introduced) (GenBank TM accession number AB010072). The sequence of P. abyssi was obtained from the Genoscope web site. ABC10α orthologs of Archaeoglobus fulgidus (33), Pyrococcus horikoshii (34), B. amygdali, and Methanococcus jannaschii (35) were and discarded using TblastN 2.0 run on the same server and data base as indicated above. The TPR plots in Fig. 5 display a function that indicates the fit to a TPR consensus sequence matrix extracted from 200 TPR units of S. cerevisiae proteins. Peaks are localized at the center of the TPR units.

RESULTS

Δ131 Interacts with the Shared RNA Polymerase Subunit ABC10α—The interaction of Δ131 with subunits of the yeast RNA Pol III was explored using the two-hybrid assay. The Δ131 gene (TFC4/YGR047c), fused in frame with the GAL4 activation domain was challenged with the complementary fusions of 12 Pol III subunits, C160, C128, C82, C53, CA40, C31, CA19, ABC27, ABC23, ABC14.5, ABC10α, and ABC10β, fused with GAL4 DNA-binding domain. The C34 subunit was not tested since it behaves, by itself, as a strong transcriptional activator (19, 36). The C25 subunit (YKL1/RPC7) (37) was not assayed. Two additional subunits, C174 and C11, have been assayed independently and gave a negative two-hybrid interaction with Δ131. Of all the Pol III subunits tested with Δ131, only ABC10α (RPC10/VHR143wa) (38) gave a positive interaction response (Fig. 1A). The β-galactosidase activity level obtained for this interaction was similar to that observed with the Δ131-TFIIB70/BRF1 interaction (Fig. 1A, see lanes 4 and 6) (15). An interaction was previously noted between C53 and a fragment of r131 (39). This interaction could not be detected using the entire Δ131 protein. Other components of the Pol III transcription system, TFIIIA, r138 and TBP, were also tested and gave negative results (not shown).

A number of deletion mutants of Δ131 were assayed in order to map the interaction domain. As shown in Fig. 1B, the Δ131α–131 interaction could not be restricted to a given subdomain of r131. Interestingly, however, some deletion mutant forms of Δ131, Δ131-TPR1, Δ131-TPR2, and Δ131-ΔTPR3 (15) were found to interact more efficiently with ABC10α than the wild-type Δ131 protein. The β-galactosidase activity generated by the Δ131-ΔTPR2-ABC10α interaction was increased 3-fold relative to ABC10α–r131. The interaction of the same collection of Δ131 mutants with TFIIB70/BRF1 (15) and TFIIB90/BRF1 (16) has been previously described. The results, summarized in Fig. 1B, show that the interaction of Δ131 variants with the three proteins was quantitatively and qualita-

1 H. Dunnay and C. Mark, unpublished observations.
2 M. L. Ferri, personal communication.
3 S. Chédin, personal communication.
tively different. First, the N-terminal part of τ131 interacted specifically with TFIIIB70/BRF1. In contrast, the deletion of the first, second, or third TPR units resulted in a decrease or no change in interaction with ABC10α. Similarly, the DTPR1 and DTPR3 mutations abrogated and decreased, respectively, the interaction of τ131 with TFIIIB90/B0, whereas the DTPR2 mutation strongly stimulated this interaction, similar to the case of ABC10α.

Altogether, these results give weight to the observed ABC10α-τ131 interaction and suggest that a conformational change of τ131 favors this interaction.

To confirm the two-hybrid results, a partially purified rABC10α-thioredoxin fusion protein was subjected to SDS-PAGE, transferred to a membrane, denatured, renatured, and probed with 35S-τ131 protein and then with antibodies directed to the T7-Tag® epitope present at the N terminus of rABC10α. As shown in Fig. 2, the 35S-τ131 probe was specifically retained at the level of rABC10α-thioredoxin fusion protein (lane 2) but not by the thioredoxin alone (lane 1). In addition, no signal was observed with a control E. coli protein extract (lane 3) or when the filter was incubated with another 35S-labeled TFIIIC subunit, τ55, used as a control (data not shown). A similar signal was observed with the mutant protein 35S-τ131-DTPR2 (lanes 4–6), which led to a thermosensitive phenotype (26). These short deletions were previously shown to confer a lethal phenotype (26). The corresponding fusion proteins were normally expressed in vivo suggesting that the lethality did not arise from mutation-induced protein degradation (results not shown). Remarkably, all three deletions were found to abolish the two-hybrid interaction with τ131. Double or single point mutations in the basic C-terminal part of ABC10α (mutants rpc10-30 and rpc10-24) that led to a thermosensitive phenotype (26) also suppressed or weakened the

**Fig. 1. In vivo interaction of wild-type or mutant τ131 proteins with ABC10α.** The two-hybrid system was used to monitor protein-protein interactions between τ131 and ABC10α. Transcriptional activation of the lacZ reporter gene was assayed by growing the transformed cells on selective medium and overlaying them with X-gal agar. A. RPC82, TFC4, and TFC4-DTPR2 were fused in frame with GAL4 activation domain sequence in pACT2 vector; RPC82, RPC10, and BRF1 were fused in frame with GAL4 DNA binding domain sequence in pAS2 vector. For each two-hybrid experiment the bait and prey are indicated by plus signs and three independent transformants are shown. β-Galactosidase dosages are indicated below cell patches; units are expressed in nanomoles of X-gal hydrolyzed per min and per mg of protein; three independent experiments were compiled for each quantification. Columns 1–3, negative controls; column 4, ABC10α-τ131 interaction; column 5, ABC10α-τ131-DTPR2 interaction; column 6, τ131-TFIIIB70/BRF1 interaction used as a reference (15). B, two-hybrid interactions between ABC10α and τ131 deletion mutants. Wild-type or mutant τ131 proteins were fused to the GAL4 activation sequence domain in pACT2 vector; ABC10α was fused with the GAL4 DNA binding domain sequence in pAS2 vector. Arbitrary values are given for white (−) and for different degrees of blue coloration (+, ++, and ++++) of cell patches on X-gal plates (same representation as in Ref. 18 and modified after Ref. 15). The results of two-hybrid interactions with TFIIIB70/BRF1 (15) and TFIIIB90/B0 (16) are given for comparison with ABC10α.
interaction with r131. On the other hand, a double mutation lying outside this region, rpc10-11, which also caused a thermosensitive phenotype (26), did not affect the two-hybrid interaction with r131. These data suggest that r131 interacts with the C-terminal part of ABC10α and point to a critical role of the conserved Arg-60 residue in this interaction.

Interestingly, as shown in Fig. 3C, the ΔTPR2 mutation increased nearly 3-fold the interaction with the wild-type ABC10α as well as with the two mutant proteins rpc10-11 and rpc10-30. In fact, the decrease of interaction strength caused by the rpc10-30 mutation (about 2-fold) was more than compensated by using the ΔTPR2 version of r131. These results confirmed that the mutant r131-ΔTPR2 protein interacted more strongly with ABC10α than with the wild-type protein.

**r131-ΔTPR2 Is an Allele-specific Suppressor of rpc10-30 Mutant**—To assess the functional role of the ABC10α-r131 interaction, we tested whether r131 or its ΔTPR2 version could rescue the ts phenotype of two ABC10α mutants that affected (rpc10-30) or did not affect (rpc10-11) the level of interaction with r131. First, overexpression of the wild-type protein r131 did not suppress these two mutations; in contrast, however, overexpression of the mutant r131-ΔTPR2 selectively suppressed the rpc10-30 mutation (Fig. 4). After 5-FOA induced loss of the high copy number plasmid harboring r131-ΔTPR2, no cell growth could be observed at the restrictive temperature, thus confirming the suppression by the ΔTPR2 mutation. The other mutation, rpc10-11, that did not affect the interaction with r131 (see Fig. 3C) was not suppressed by r131-ΔTPR2. Therefore, the mutation r131-ΔTPR2 restored both the two-hybrid interaction with rpc10-30 and the growth of the rpc10-30 mutant at restrictive temperatures. The fact that this suppression was not observed with the wild-type r131 protein could be explained in the light of the β-galactosidase induction level. Indeed, the level of interaction of the ABC10α-r131-ΔTPR2 couple was nearly 3-fold that of the ABC10α-r131. Note that the rpc10-30-r131-ΔTPR2 interaction was also stronger than that of the two wild-type proteins (see Fig. 3C).

We also checked whether ΔTPR2 mutation was able to suppress a number of already described mutations in the Pol III transcription system. The following mutations, affecting the C160, C31, and C53 subunits of Pol III were tested: rpc160-270 (20), rpc160-112 (27), rpc31-236 (20), and rpc53/256/424 (28) (Fig. 4). None of these ts mutations was found to be suppressed at non-permissive temperature by overexpression of r131-ΔTPR2, thus supporting the allele specificity of the rpc10-30 suppression.

It should be noted that the ΔTPR2 mutation has been previously reported to confer a lethal phenotype to yeast cells harboring a partially deleted copy of TFC4 (15). However, we found that in another genetic context, in which the r131 gene has been totally deleted, the same mutation turned out to be viable but conferred a thermosensitive phenotype (data not shown). It was intriguing that the rpc10-30 ts mutant could be rescued at non-permissive temperature by the overexpression of r131-ΔTPR2 which also caused a ts phenotype. Note, however, that this suppression experiment was performed in yeast cells harboring a wild-type copy of TFC4. When r131-ΔTPR2 was overexpressed in a wild-type context for both ABC10α and r131, no effect on the cell growth rate could be observed, indicating that the ts phenotype of r131-ΔTPR2 was not dominant (not shown).

**Putative Archaeal Orthologs of ABC10α and r131**—Among the five subunits common to the three nuclear RNA polymerases, ABC27, ABC23, and ABC10α have an archaeal counterpart, named H, K, and N (40). No archaeal ortholog has yet been described for the ABC10α subunit. As five complete archaeal genomes are available, it was of interest to search for a possible counterpart of the eukaryotic ABC10α subunit. By using the sequence of ABC10α of S. cerevisiae as entry and TblastN 2.0.8 (32), a small unannotated ORF, named AF0055, was identified in the genome of A. fulgidus (33). Using this ORF as a probe, a similar ORF was identified in P. horikoshii (34) and P. abyssi genomes. By using the P. abyssi ORF, a similar ORF was also found in the M. jannaschii genome (35). Remarkably, these four short ORFs are always found immediately 3’ of the gene coding for L37α, a conserved ribosomal protein specific to archaea and eukarya. Archaeal operons containing RNA polymerase subunit genes often contain ribosomal protein genes (40). The P. horikoshii sequence has been reported as being homologous to an unspecified S. cerevisiae RNA polymerase subunit, however, with a wrongly estimated length making this ORF overlap the end of L37α protein (34). A closer examination of the Methanobacterium thermoautotrophicum genome (41) revealed a similar short ORF located 3’ of the L37α protein gene but lacking an initiation codon. These five archaeal sequences are shown in Fig. 5, alongside with the ABC10α sequences of A. thaliana, Caenorhabditis elegans, Homo sapiens, C. albicans, Schizosaccharomyces pombe, and S. cerevisiae. These sequence comparisons strongly suggest the existence of an ortholog of ABC10α in archaea.

A protein (MJ0941) of the archaea M. jannaschii has been annotated as a putative subunit of transcription factor IIIC (35). This observation was intriguing and prompted us to reexamine the relationship of this protein to TFIIIC subunits. Indeed, the archaeal protein showed a clear sequence similarity to r131. However, r131 and its human counterpart are characterized by their high content in TPR motives clustered in three blocks of 5, 4, 1, and 1 TPR (Fig. 5B). As the archaeal ORF was made of a succession of 9 TPR motives, the similarity between the two proteins was essentially based on the presence of the TPR motives (Fig. 5B). Furthermore, the archaeal protein was much shorter than r131; it was a tandemly duplicated protein, and the same arrangement was not conserved in other archaeal...
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**DISCUSSION**

We present biochemical and genetic evidence in favor of a functional contact between Pol III and its cognate assembly transcription factor TFIIIC via a direct interaction between r131 and a shared polymerase subunit ABC10α. This interaction extends the role of TFIIIC beyond its known functions in promoter recognition and TFIIIB assembly.

The two-hybrid and far Western experiments clearly demonstrated the affinity of ABC10α for r131. A deletion mutant analysis could not restrict the interaction with ABC10α to a specific domain of r131. The binding of ABC10α might necessitate a cooperation between two or more domains of r131. The stronger interaction of the r131-ΔTPR1, ΔTPR2, or ΔTPR3 mutants over the wild-type protein suggests that a conformational change, facilitated by the absence of TPR motif 1, 2, or 3, exposes some important interacting domain. In two-hybrid experiments, r131-ΔTPR2 interacted more strongly with TFIIIB90/BRF1 (16), which was not the case with TFIIIB70/BRF1 (15). Remarkably, in a random mutagenesis experiment carried over TPR units 1–8, the search for suppressors of an A block-down mutation yielded 10 mutants covering a 53-amino acid area extending over TPR1, -2, and -3 and centered on TPR2. One of these mutants, PCF1-2, was shown to activate Pol III transcription by increasing the recruitment of TFIIIB70/BRF1 through a non-equilibrium binding mechanism (42). These results and those presented in this work converge to underscore the importance of the second TPR motif. It is possible that both the improved recruitment of TFIIIB70/BRF1 by PCF1-2 and the better interaction between r131 and ABC10α could stem from the same conformational change in r131 favored either by mutations that disrupt the second TPR structure or by the deletion of this whole TPR unit. In fact, drastic conformational changes are likely to occur in r131 during the TFIIIB assembly process (43).

The finding of an interaction between two proteins belonging to two different multiprotein complexes raises the question of its functional significance. The ABC10α-r131 interaction occurred via the C terminus domain of ABC10α and was affected by a thermosensitive mutation in that region, rpc10-30. Interestingly, the ts phenotype of the rpc10-30 mutant was suppressed by overexpression of the r131-ΔTPR2 protein as could be expected since the ΔTPR2 mutant interacted more strongly with ABC10α. As a matter of fact, we observed that a ts mutation (rpc10-11) in another region of ABC10α did not impair genomes. Therefore, we found no evidence for the presence of TFIIIC-related proteins in archaea.

**Fig. 3.** Two-hybrid interaction of mutants ABC10α with r131 or r131-ΔTPR2. A, the sequences of wild-type (48) and mutant (26) ABC10α proteins are shown; asterisks indicate stop codons. Bold and capitalized letters have the same meaning as in Fig. 5A. B, the phenotype of the ABC10α mutants is summarized (26) as follows: +, wild type; −, lethal; ts, thermosensitive; Pol III or Pol, specific transcription defect in vivo (26). The level of two-hybrid interaction is indicated as in Fig. 1B. C, effect of the ΔTPR2 mutation on the two-hybrid interaction with ABC10α mutants. The strength of two-hybrid interaction between r131 (TFC4) or mutant r131-ΔTPR2 with wild-type (RPC10) or mutant ABC10α (rpc10-30 or -11) was evaluated by β-galactosidase dosage; units are expressed in nanomoles of X-gal hydrolyzed per min and per mg of protein. Black bars denote combinations involving r131 or mutant r131-ΔTPR2 and ABC10α or rpc10-30 mutant. Positive (TFC4 × BRF1) and negative (RPC82 × RPC10, ΔTPR2 × RPC82, etc.) controls are shown for comparison.

**Fig. 4.** Allele-specific suppression of rpc10-30 by ΔTPR2 mutation. Strains YLR-06 and YLR-03 carrying the rpc10-30 and rpc10-11 ABC10α mutations were transformed with plasmids, pFLRPC10, pFL-r131, and pFLΔTPR2 overexpressing ABC10α, wild-type r131, and r131-ΔTPR2, respectively, as indicated. The empty vector pFL44 was used as a control. Overexpression of r131-ΔTPR2 allowed cell growth of the ABC10α rpc10-30 but not of rpc10-11 mutant. Four Pol III mutant strains were checked for their ability to be rescued by r131-ΔTPR2 as follows: MW670 (rpc160–112), MW1029 (rpc160–270), SC91 (rpc53–256/424), and D132-ID (rpc31–236). Transformants were streaked on YPD medium and grown at the permissive (30 °C) or non-permissive (37 °C) temperature for 4 days.
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The two-hybrid interaction with r131, whereas the thermosensitive mutant (rpc10-30) in the C-terminal region affected the interaction. Reciprocally, the overexpression of wild-type r131 or rpc10-ΔTPR2 was unable to suppress the rpc10-11 mutant that did not affect the ABC10α-r131 interaction. It is also important to note that the rpc10-30 mutant was specifically affected, in vivo, in Pol III transcription, whereas the nonrescueable rpc10-11 mutant was not Pol III-specific (26). ABC10α has been previously identified as a suppressor of tsv115, a mutation in the r138 subunit of TFIIIC (44) affecting TFIIIC-DNA binding and the assembly of the Pol III preinitiation complex (45). None of the other Pol III subunits tested at that time (C160, C128, C82, C53, AC40, C34, C31, AC19, and ABC10β) were found to suppress the tsv115 mutation when overexpressed. ABC10α was suggested to be a critical subunit limiting the rate of Pol III assembly. In fact, a purified Pol III harboring the rpc10-30 mutation did not display any transcriptional defect in vitro, but the level of Pol III in mutant extracts was much decreased (26). Diploid cells with only one gene copy for ABC10α have a growth defect, ABC10α is indeed likely to affect a rate-limiting step in polymerase assembly (26). It remains that the Pol III-specific phenotype of rpc10-30 may be due in part to its deficiency in TFIIIC interaction since it is partially suppressed by r131-ΔTPR2.

A contact between yeast TFIIIC and Pol III is not implied in the sequential initiation complex assembly model where TFIIIC assembles TFIIIB which in turn recruits Pol III. Indeed a preassembled TFIIIB-DNA complex can direct accurate transcription by Pol III in the absence of TFIIIC (46). Nevertheless, the association of Pol III with TFIIIC in yeast extracts has been demonstrated by communoprecipitation experiments (18). Therefore, the ABC10α-r131 interaction may be involved in the formation and/or stability of a Pol III holoenzyme. Alternatively, this interaction may facilitate the recruitment of Pol III by the TFIIIB-TFIIIC-DNA complex. An interaction of Pol III with TFIIIC is also likely to occur when the enzyme elongates through the TFIIIC-bound intragenic promoter. Therefore, Pol III may well engage in many interactions with TFIIIC which are clearly homologous to r131, with its characteristic TPR motifs.

B S. cerevisiae r131 and putative archaeal ortholog

The amino acids equivalence used are: D and E; I, L, and V; G and S; K and R; and F and Y. Gaps, indicated as dashes at some positions in the N-terminal region. The amino acids conserved separately in eukaryotic or in archaeal sequences, and indicate amino acids conserved in both eukaryotic and archaeal sequences (the sequence of M. thermoaotrophicum that departs from the other ones was not taken into account at some positions in the N-terminal region). The amino acids equivalence used are: D and E; I, L, and V; G and S; K and R; and F and Y. Gaps, indicated as dashes, were introduced to maximize homologies.

The presence of TPR motifs is indicated by the peaks localized at the center of each TPR motif which are numbered from 1 to 11 in r131 sequence or 1 to 9 in MJ0941.

The sequence of M. thermoaotrophicum ortholog ORF is found immediately 3’ of the L37A ribosomal protein gene (the distance from the stop codon of L37A is indicated at the left). Note that the sequence of M. thermoaotrophicum lacks an initiation codon. The position of the stop codons in the genomes and orientation of the ORFs are indicated at the right. Capital letters indicate amino acids conserved separately in eukaryotic or in archaeal sequences, and boldface capital bold letters indicate amino acids conserved in both eukaryotic and archaeal sequences (the sequence of M. thermoaotrophicum that departs from the other ones was not taken into account at some positions in the N-terminal region). The amino acids equivalence used are: D and E; I, L, and V; G and S; K and R; and F and Y. Gaps, indicated as dashes, were introduced to maximize homologies. # and ~ denote residues strictly or partly conserved, respectively. B, comparison of r131 and its putative archaeal ortholog from M. jannaschii (ORF MJ0941). The presence of TPR motifs is indicated by the peaks localized at the center of each TPR motif which are numbered from 1 to 11 in r131 sequence or 1 to 9 in MJ0941.

FIG. 5. Sequence comparison of eukaryotic ABC10α and r131 and their putative archaeal orthologs. A, six eukaryotic ABC10α orthologs are displayed: A. thaliana, C. elegans (49), H. sapiens (31), C. albicans, S. pombe (31), and S. cerevisiae (38). A putative ortholog of ABC10α was found in each of the five complete archaeal genome available: A. fulgidus (33), P. horikoshii (34), P. abyssi (see “Experimental Procedures”), M. jannaschii (35), and M. thermoaotrophicum (41). In all five genomes, the ABC10α ortholog ORF is found immediately 3’ of the L37A ribosomal protein gene (the distance from the stop codon of L37A is indicated at the left). Note that the sequence of M. thermoaotrophicum lacks an initiation codon. The position of the stop codons in the genomes and orientation of the ORFs are indicated at the right. Capital letters indicate amino acids conserved separately in eukaryotic or in archaeal sequences, and boldface capital bold letters indicate amino acids conserved in both eukaryotic and archaeal sequences (the sequence of M. thermoaotrophicum that departs from the other ones was not taken into account at some positions in the N-terminal region). The amino acids equivalence used are: D and E; I, L, and V; G and S; K and R; and F and Y. Gaps, indicated as dashes, were introduced to maximize homologies.
tion of TFIIIC subunits with Pol III subunits suggests additional functions for TFIIIC.

Acknowledgments—We thank J.-M. Buhler for the gift of plasmid pRESEThisRPC10, O. Lefebvre for helpful comments, and P. Thuriax for helpful discussions and a critical reading of the manuscript. Sequencing of C. albicans was accomplished with the support of the NIDR and the Burroughs Wellcome Fund.

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