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A protein–protein interaction map of yeast RNA polymerase III

(*Saccharomyces cerevisiae*/two-hybrid system)

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ABSTRACT **The structure of the yeast RNA polymerase (pol) III was investigated by exhaustive two-hybrid screening using a library of random genomic fragments fused to the Gal4 activation domain. This procedure allowed us to identify contacts between individual polypeptides, localize the contact domains, and deduce a protein–protein interaction map of the multisubunit enzyme. In all but one case, pol III subunits were able to interact *in vivo* with one or sometimes two partner subunits of the enzyme or with subunits of TFIIIC. Four subunits that are common to pol I, II, and III (ABC27, ABC14.5, ABC10α, and ABC10β), two that are common to pol I and III (AC40 and AC19), and one pol III-specific subunit (C11) can associate with defined regions of the two large subunits. These regions overlapped with highly conserved domains. C53, a pol III-specific subunit, interacted with a 37-kDa polypeptide that copurifies with the enzyme and therefore appears to be a unique pol III subunit (C37). Together with parallel interaction studies based on dosage-dependent suppression of conditional mutants, our data suggest a model of the pol III preinitiation complex.**

Eukaryotic transcription is mediated by large multiprotein complexes in which each of the three nuclear RNA polymerases (pols) interact with their cognate preinitiation factors. The pols themselves have been well characterized in terms of subunit composition, especially in the case of the yeast *Saccharomyces cerevisiae*. However, the spatial organization of the enzyme subunits and the way they interact with preinitiation complexes or with other components of the yeast nucleus are still poorly understood. Electron microscopy so far has provided the most accurate structural description of the *Escherichia coli* enzyme (1) and of yeast pol I (2, 3) and II (refs. 4–6 and references therein), revealing a striking similarity in the overall shape of these enzymes. In the case of yeast pol I, six subunits (or domains thereof) were localized by immunoelectron microscopy of antibody-labeled enzymes (2, 7). Site-specific protein-DNA crosslinking also shed light on the general architecture of pol II (8, 9) and III (10–12) transcription complexes.

These studies are still far from providing a comprehensive picture of the structural organization of the eukaryotic pols. Alternatively, each subunit can be tested for its ability to selectively associate with other subunits of the same heteromultimeric complex. In the case of human pol II, an *in vitro* test based on glutathione *S*-transferase pull-down assays has suggested numerous contacts within the pol II complex (13). In *Schizosaccharomyces pombe*, studies based on Far Western blotting, which were in some cases supported by independent protein–protein crosslinking studies, suggested that the two large pol II subunits interact with all of the other smaller subunits (9, 14). The two-hybrid system is an alternative to biochemical methods that allows one to detect interactions between proteins in the cellular context of the yeast nucleus (ref. 15 and references therein). Previous work from our laboratory has taken advantage of this method to identify putative contacts between some subunits of yeast pol III and general transcription factors (16–19). The biological relevance of the interactions was buttressed by independent evidence. For example, subunits AC19 and AC40 also are related by mutual dosage-dependent suppression effects (17) and were shown to colocalize by immunoelectron microscopy (2). Furthermore, the interaction between the C34 pol III-specific subunit and the TFIIIB70 component of the TFIIIB general transcription factor was shown to be essential for the recognition of the preinitiation complex by the enzyme and for the formation of the open complex (20). Encouraged by these results, we have used a systematic two-hybrid screen in which each individual cloned pol III subunit was screened against a yeast genomic library (15) where gene fragments of 700-bp mean size are randomly fused to the Gal4p activation domain ($Gal4p_{AD}$). These data yielded an interaction map of the pol III subunit complex.

MATERIALS AND METHODS

Media, Strains, and Plasmids. Yeast genetic techniques and media have been described by Sherman (21). All two-hybrid tests were performed in strain Y190 [*MAT**a** gal4 gal80 his3 trp1–901 ade2–101 ura3–52 leu2–3, 112 URA3*::*GAL1*::*lacZ LYS2*::*GAL4(UAS)*::*HIS3 cyh*^{R}; ref. 22].

Fusions used as "baits" contained the complete ORF of the relevant polypeptide fused to the Gal4p DNA binding domain ($Gal4p_{BD}$) cloned in pAS2ΔΔ (15) or pGBT9 (23) via a unique restriction site placed just upstream of the translation intiation codon. Most baits were expressed from the pASΔΔ vector, but some fusion proteins were toxic, in which case we used the pGBT9 vector that produces lower amounts of the fusion protein (24). Fusion joints as well as the complete ORF of the PCR-generated fusions were sequenced. In the case of C160, AC40, AC19, ABC14.5, ABC10α, ABC10β, and TFIIIB70, the *GAL4(1–147)*-pol gene fusions were tested for complementation of a corresponding null allele in a plasmid shuffle assay. Only *GAL4(1–147)-RPB10*, encoding ABC10β, was unable to functionally replace the wild-type gene. ABC10β and C128 correct expression were tested by Western blotting. The C82 fusion had been shown previously to be functional in the two-hybrid assay (16).

Two-Hybrid Screening and Identification of Interacting Proteins. The FRYL genomic library (15) contained randomly sheared genomic DNA fragments of 700-bp mean size in a

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Abbreviations: pol, RNA polymerase; $Gal4p_{BD}$, Gal4p DNA-binding domain; 3AT, 3-amino-triazole.

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modified pACT2 vector. The number of clones is 5.0×10^6, meaning that a fusion occurs every 4 nt of the yeast genome sequence and thus theoretically permits the identification of all possible interactions provided a sufficient number of clones is tested. The library DNA was purified by ultracentrifugation in a CsCl gradient to transform strain Y190 containing the bait fusion by the lithium acetate method (25). Transformed cells were directly plated on SD adenine (100 μg/ml) minimal medium plates containing 10, 25, or 50 mM 3-amino-triazole (3AT). Colonies growing after 4–7 days were streaked on SD + adenine + histidine and assayed for β-galactosidase production in an overlay plate assay. The intensity of the coloration was calibrated by comparison with known pairs of interactors in which the β-galactosidase activity had been measured previously (16). Blue colonies were restreaked on the same medium and tested again for β-galactosidase production. Prey plasmid DNA was recovered and transformed in *E. coli* strain 1066 [*trpC-9830 leuB-6 pyrF-74*::*Tn5* Δ*(lac I POZYA)-74 galU galK hsdR rpsL*] selecting the yeast *LEU2* marker. Alternatively, we prepared total yeast DNA by using a QIAamp kit (Qiagen, Chatsworth, CA), amplified the prey DNA by PCR, and purified the product on a QIAquick column. After sequencing, the identity of the insert was determined by using the Saccharomyces Genome Database Blast service (http://genome-www2.stanford.edu/cgi-bin/SGD/nph-blast2sgd).

RESULTS

General Approach. Seventeen complete ORFs corresponding to 15 cloned subunits (of 17) of pol III and the TFIIIB70 and TATA box-binding protein (TBP) components of TFIIIB were fused to the Gal4p_{BD} in the pAS2ΔΔ or pGBT9 vector and introduced into the tester strain Y190, which has two reporter genes for two-hybrid interaction, *GAL1*::*lacZ* and *GAL(UAS)*::*HIS3*. The transformed strains were plated on 10, 25, or 50 mM 3AT minimal medium to detect background activation of the *GAL(UAS)*::*HIS3* reporter gene. Fusions with the C31, C34, and TBP proteins conferred resistance to 50 mM 3AT, indicating that they operate as transcriptional activators of pol II, and thus could not be used in the screen. The 14 remaining fusions were tested against the DNA genomic library of Fromont-Racine *et al.* (15), by using a two-step screening procedure based on cell growth in the presence of 3AT and β-galactosidase activity, as described in *Materials and Methods*. This analysis allowed us to identify putative partnerships by using full-length proteins as baits and random fragments of a mean size of 230 aa as preys. This approach is distinct from a previous one where complete ORF fusions of individual subunits were tested against each other, which revealed subunit-subunit interactions involving AC19 and AC40, the two subunits shared by pol I and III (17), and a triad of pol III subunits (C82, C34, and C31; ref. 16), and also identified C34 and the τ131 subunit of TFIIIC as partners of the TFIIIB70 component of the initiation factor TFIIIB (16, 18). As discussed below, most of these interactions were found again in the present work.

Table 1 summarizes the outcome of our two-hybrid screens. Except for the two large subunits of pol III (C160 and C128) and the common subunit ABC23, which yielded no positive clone, a large number of plasmids was selected in the other screens. The absence of interacting clones in the C160 and C128 screens did not stem from a low expression level because the fusion proteins were detected by Western blot. Moreover, the *GAL4(1–147)-RPC160* gene fusion complemented a null mutation in the *RPC160* gene, indicating that the fusion protein functioned correctly. This behavior need not be the consequence of the size of the two large subunits, because some large proteins have been screened successfully with the same FRYL library (unpublished results and M. Fromont-Racine, personal communication). Except for C25, the remaining screens always yielded at least one component of the pol III complex, which are dealt with in more detail in this report. In all cases, many of the clones isolated corresponded to gene products that were unrelated to known components of the transcription complexes but might in some cases reveal connections between transcription and other aspects of being nuclear metabolism.

Interactions with the Common Subunits ABC27 (Rpb5), ABC14.5 (Rpb8), ABC10α (Rpb12), and ABC10β (Rpb10). Five small subunits are shared between the three forms of pols (26). The four subunits that were successfully screened (ABC27, ABC14.5, ABC10α, and ABC10β) yielded positive clones that corresponded to fragments of the largest and/or second largest subunit of pol I, II, or III (Table 1). Seventeen independent clones (some of which were found several times) that were obtained with the Gal4p_{BD}-ABC27 fusion encoded fragments of the largest subunit of pol I, II, or III (Table 1 and Fig. 1). All of these fragments overlapped, restricting the interacting domain to the 1523–1576 amino acid interval of

Table 1. Summary of the two-hybrid screens

Bait	Transformants tested ($\times 10^{-6}$)	$3AT^R$ β-Gal$^+$ clones	Clone sequenced	Transcription complex subunit*
C160	3.0	0	0	None
C128	6.5	0	0	None
C82	24.0	72	39	C34 (1, 1)
C53	13.6	55	38	TFC4 (1, 1); C37 (15, 4)
AC40	50.4	235†	109	AC19 (19, 4); A135 (4, 2)
ABC27	7.2	207	118	A190 (12, 10); B220 (2, 1); C160 (7, 6)
C25	15.0	111	71	None
ABC23	5.5	0	0	None
AC19	38.3	450†	153	AC40 (6, 5); A135 (3, 2); B44 (2, 2)
ABC14.5	12.0	31	29	B220 (1, 1); C160 (6, 4)
C11	10.6	101	83	C128 (2, 2)
ABC10α	7.3	196	122	A135 (1, 1)
ABC10β	113.2	276	209	B220 (1, 1); B150 (2, 1)
TFIIIB70	9.2	96	82	TFC4 (2, 2)
A12.2	11.0	105	82	A135 (1, 1)
B12.5	19.3	302	185	B44 (27, 18)

*The numbers between the parentheses represent the total number of selected clones and the number of independent clones, respectively.

†Only 320 of the 968 $3AT^R$ clones selected in the AC40 screen and 632 of the 2,136 $3AT^R$ clones selected in the AC29 screen were tested for β-galactosidase production.

A190, the 1170–1406 interval of B220, and the 1274–1381 interval of C160. These protein domains overlap with the conserved domain **h** and define a specific domain of contact between the large subunits of pols and ABC27.

A similar situation was observed for ABC14.5 where overlapping clones delimited the amino acid sequence from residue 455 to 705 of the pol III large subunit (C160) and from residue 516 to 639 of its pol II counterpart (B220; Fig. 1). Again, these intervals overlap with conserved domains, corresponding in this case to regions **d** and **e**. Because the corresponding domains of pol I large subunit were not isolated, we constructed a fusion of the corresponding fragment of A190 (663–805) and the C160 (547–697) fragment homologous to the interacting region of B220. Both fusions gave a strong positive response when tested against the Gal4p_{BD}-ABC14.5 bait (Fig. 1).

In the case of the Gal4p_{BD}-ABC10β fusion, three clones corresponded to a fragment of the largest (B220, amino acids 230–376) and second largest (B150, amino acids 1075–1201) subunit of pol II. However, the fusions including the equivalent region from C160 and A190 large subunits did not interact, nor did extended regions beginning just after conserved region **a** and ending just before region **d** [C160 (144–462); A190 (139–577)]. The B150 fragment included two regions: region I, which belongs to the active site of the enzyme, and the adjacent zinc finger (27, 28). Subcloning experiments showed that the zinc finger domain [B150 (1150–1202)] interacted weakly with ABC10β. However, ABC10β did not interact with the homologous region of C128 (1083–1127), but did with the two fragments of A135 (pol I) that were selected in the AC40 and AC19 screens (see below; Fig. 2*A*).

One of the 122 plasmids selected in the ABC10α screen turned out to encode the amino acid sequence extending from amino acid 670 to 1144 of A135, the second largest subunit of pol I (Fig. 2*A*). An overlapping clone of A135, isolated in the AC19 and AC40 screens (see below), also interacted with ABC10α. No insert coding for the corresponding subunits of pol II or III were found. The homologous fusions were constructed (intervals 654-1149 of C128 and 679-1224 of B150) but did not interact with Gal4p_{BD}-ABC10α.

Interactions with the AC40 and AC19 Subunits Shared by Pol I and III. AC19 and AC40 subunits previously were shown to interact with each other in the two-hybrid system (17). AC40 has significant homology with the α subunit of the bacterial enzyme. AC19 also may be related to α. Pol II has two subunits, B44 (Rpb3) and B12.5 (Rpb11), which are closely related to AC40 and AC19, respectively. Nine independent clones obtained with the Gal4p_{BD}-AC19 fusion coded for the pol subunits AC40 (five clones), B44 (two clones), and A135 (two clones). As shown in Fig. 2*B*, the AC40 and B44 sequences all overlap with the C-terminal half of these proteins (amino acids 175–335 and 181–318, respectively). A parallel screen performed with the B12.5 subunit of pol II selected 27 clones coding for 18 independent carboxyl-terminal fragments of B44, but no clone coding for AC40 was found (Table 1). The interactions between AC19 and AC40 and between B12.5 and B44 thus involve a very conserved interface. Finally, two overlapping clones encoding the C-terminal end of A135 were selected in the AC19 screen (amino acids 670-1144 and 678-1055). The longest clone was identical to that isolated in the ABC10α screen but the short one also interacted when tested with ABC10α. As in the case of ABC10α, the corresponding C128 (654–1051) and B150 (679–1119) fusions did not interact detectably with AC19.

In the case of AC40, we obtained four different AC19-coding clones (Fig. 2C) that overlapped from amino acid 48 to the C-terminal end. This screen also yielded the same two *RPA135* clones that were isolated with AC19 and ABC10α. Again, the corresponding C128 and B150 fusions did not interact with AC40.

Screening with Pol III-Specific Subunits and TFIIIB70. Previous work showed that TFIIIB70 interacts with the τ_{131} subunit of TFIIIC and the C34 subunit of pol III (16, 18, 29). These interactions are important for the recruitment of TFIIIB by TFIIIC (18, 29) and of pol III by the preinitiation complex (16, 20). Two different clones coding for τ_{131} were isolated with

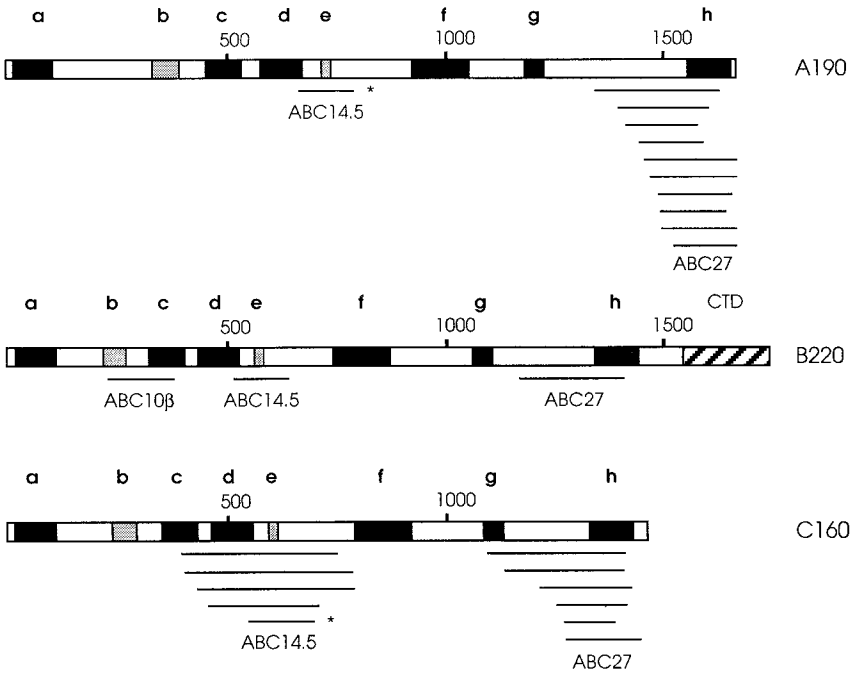

FIG. 1. Mapping of the interaction domains between the β'-like subunits and the common subunits. The large subunits (A190, B220, and C160) are represented by rectangles and are drawn to scale. The black boxes indicate the conserved regions, more divergent regions are represented by gray boxes (26). The scale and the nomenclature of the regions are indicated above the boxes. Individual clones coding for fragments selected by the ABC27, ABC14.5, and ABC10β screens are represented by horizontal lines. The asterisks indicate the A190 and C160 clones that were constructed to test the interaction of restricted domains with ABC14.5.

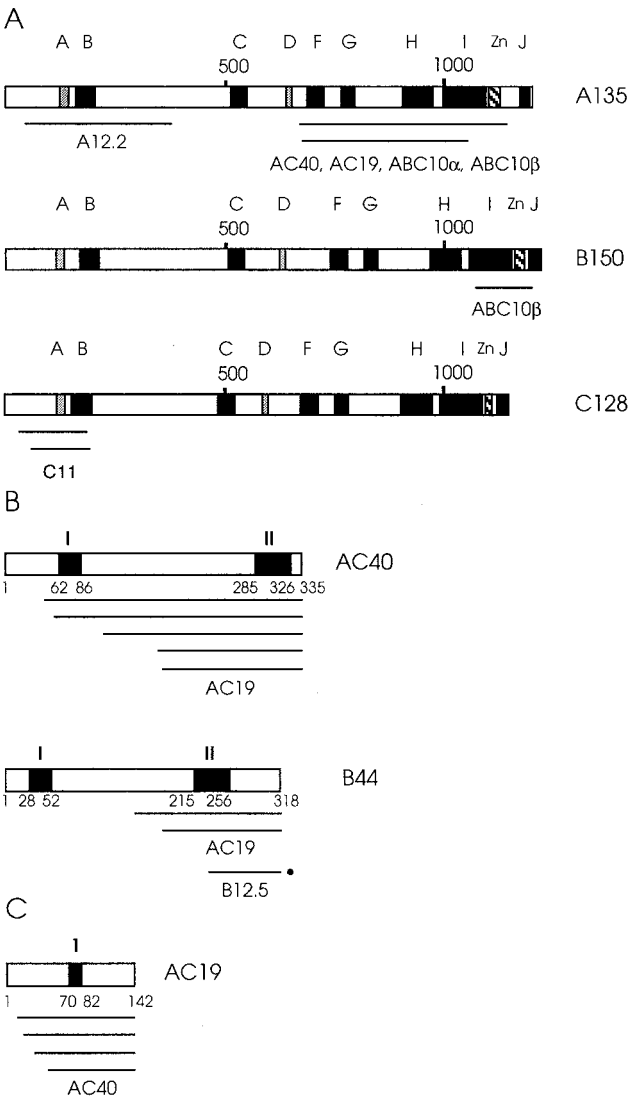

FIG. 2. Mapping of the interactions with AC40, AC19, ABC10α, and ABC10β. (*A*) Interaction of AC40, AC19, ABC10α, and ABC10β with the β-like subunits. The β-like subunits (A135, B150, and C128) and the corresponding regions are represented as in Fig. 1. Zn indicate the zinc finger region of A135, B150, and C128. (*B*) AC40 and B44 domains interacting with AC19. The black dot indicates that only the shortest of the 18 independent B44 fragments selected in the B12.5 screen was represented. (*C*) AC19 protein sequences selected in the AC40 screen.

TFIIIB70; the short one encompassed the amino acid sequence of τ_{131} from residues 12-278, in good agreement with the previously mapped domain of interaction (18).

Yeast pol III contains several specific subunits, four of which (C82, C53, C25, and C11) were screened (the other two, C34 and C31, activated transcription when fused to $Gal4p_{BD}$). The C25 screen yielded 71 clones belonging to 44 different genes but none coded for a pol III or transcription factor subunit. No C31- or C34-encoding clone was selected in the FRYL genomic library when using a $Gal4p_{BD}$-C82 bait. However, one C34-encoding clone (starting at amino acid 48 to the C terminus of the protein) was found by using another library with a larger fragment mean size. It already was observed with the full-length proteins that conditional mutations in C34 abolish the interaction (16, 20). Moreover, C34 and C82 are part of a subunit subcomplex together with C31 as are their human homologues (30, 31).

The $Gal4p_{BD}$-C53 screen selected one clone encoding the 750 N-terminal aa of the τ_{131} subunit of TFIIIC. This observation suggests a possible interaction between pol III and TFIIIC via the C53 subunit. This screen also yielded four different fusions corresponding to the same ORF, *YKR025w*, encoding a polypeptide with a predicted molecular mass of 32 kDa. The domain of interaction spanned the sequence from amino acids 62 to 200. A 37-kDa polypeptide is associated with pol III (32) and can be separated from AC40 under certain electrophoretic conditions (G. Peyroche and M. Riva, personal communication). Sequences of tryptic peptides from that protein were determined and were found to be encoded by *YKR025w*. This result strongly suggested that the *YKR025w* gene product, C37, is a unique pol III subunit.

Finally, the C11 screen yielded two overlapping clones of C128, the second largest subunit of pol III. The minimal domain of interaction (30–182) included the loosely conserved region A and the N-terminal half of region B. Strikingly, the homologous pol I subunit, A12.2, yielded one clone covering the homologous region of the A135 pol I subunit, thus confirming the specificity of the interaction (Table 1, Fig. 2).

DISCUSSION

We have used a systematic and exhaustive two-hybrid screening strategy to map the interacting domains of yeast pol III subunits and TFIIIB70. These experiments have disclosed numerous protein–protein contacts, often allowed the mapping of the interacting domains, and identified a pol III subunit, C37. As discussed below, these data can be incorporated into a model of the pol III initiation complex (Fig. 3).

Several lines of evidence suggest that the interactions uncovered in the present study are specific and biologically relevant (see ref. 15 for a thorough discussion of the specificity of exhaustive two-hybrid screening). First, all but one (C25) of the 12 proteins for which interacting clones were selected were found to contact one, or in some cases two, partner subunit(s) belonging to the pol III system. Second, most individual subunits yielded several fragments of the same target subunit, which invariably corresponded to overlapping regions. Third, the interactions involving common or conserved subunits shared by pol I, II, or III, identified homologous domains in the three enzymes. This finding was particularly striking for the two common subunits, ABC14.5 and ABC27, but also for the conserved subunits C11 (pol III) and A12.2 (pol I), or AC19 (pol I and III) and B12.5 (pol II). The fact that similar interactions were invariably found between homologous domains of conserved subunits argues against fortuitous contacts. Fourth, the C53 screen illustrates the predictive power of the two-hybrid approach, because the C53 major partner was the gene product of the *YKR025w* ORF, which turned out to encode a 37-kDa polypeptide (C37) that copurifies

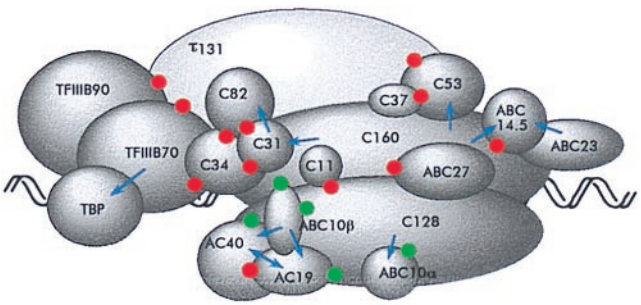

FIG. 3. A model of the pol III transcription initiation complex. Protein–protein contacts observed by using the two-hybrid system are indicated by red dots. Green dots indicate the interaction between AC40, AC19, ABC10α, and ABC10β with A190 and A135 pol I subunits homologous to C160 and C128. Genetic interactions observed by using multicopy suppression experiments of thermosensitive mutations are indicated by arrows. The arrowhead points toward the subunit harboring the mutation that was suppressed. For the sake of simplicity, only the τ131 and τ138 subunits of TFIIIC are represented.

with yeast pol III (32), therefore supporting the view that this protein is a bona fide subunit of pol III. Finally, the confidence that can be placed in screens also can be assessed by comparing our data with 56 exhaustive screens performed with the same FRYL library by using proteins not belonging to the transcription systems [European Two-Hybrid Analysis of Proteins Involved in RNA Metabolism (TAPIR) network]. In these screens, none of the interacting clones corresponded to pol subunit fragments selected here, again suggesting that the interactions are specific.

Because the two-hybrid experiments are performed in *S. cerevisiae*, an obvious concern is that some partner proteins may not contact each other directly, but could instead interact with a third subunit that would mediate the interaction. Several arguments suggested that this was not the case, except possibly for some of the interactions with A135 (see below). First, when an interaction via an intermediary protein has been observed in the two-hybrid system, it depended on the overexpression of the bridging component (33). Second, the fact that small interacting domains usually were delineated (from 52 to 161 residues) makes the existence of a third component less likely because the bridging protein should interact with the same small domain as the prey protein. Finally, the bridging protein and the one that interacts indirectly also should contact each other.

AC40, AC19, ABC10α, and ABC10β selected the same domain of A135, raising the possibility that in this case the interactions might be indirect. This was probably not the case for ABC10α because the conditional phenotype of an ABC10α mutant, defective in pol III assembly, is suppressed by the overexpression of C128 and aggravated by the overexpression of A135 or B150, whereas AC40, AC19 and ABC10β had no effect (L.R., S. Mariotte, S. Chidin, and P.T., unpublished work). Additionally, the smallest A135 interacting fragment was relatively large (378 aa) and thus conceivably could bind to several of these small subunits independently. Our data suggesting that AC40 and AC19 interact with the second largest subunit of pol I are also in agreement with the observation that limited dissociation of the *S. pombe* pol II yields a stable subcomplex containing Rpb2, Rpb3, and Rpb11 subunits (34). Contrary to our expectations based on the homology with A135, a corresponding fragment of C128 did not interact with AC40, AC19, ABC10α, and ABC10β. This finding could indicate that these subunits do not interact with pol III as they do with A135 or, alternatively, that the C128 fragment could not fold properly for two-hybrid interactions. Thus the interactions presented in the model (Fig. 3) between AC40, AC19, ABC10β, and C128 should be considered as tentative.

It is interesting to compare the outcome of the two-hybrid approach with biochemical methods that were used to explore subunit contacts in eukaryotic pols. In *S. pombe*, studies based on Far Western blot analysis indicated that Rpb5 and Rpb3 subunits (corresponding to ABC27 and B44, respectively) each contact five different pol II subunits, whereas the two large subunits were found to interact with all of the other pol II subunits, leading to the suggestion that the large subunits provide an assembly platform for the small subunits (9, 14). This multiplicity of interactions is intriguing and raises the question of the specificity of this assay. Acker *et al.* (13) investigated protein–protein interactions within the human pol II complex by using a glutathione *S*-transferase (GST) pull-down assay with pairs of subunits coexpressed in a baculovirus system. Several of the interactions observed also were found in our experiments. However, 14 of 19 detected contacts involved hRPB5 or hRPB3, suggesting that some of these interactions might reflect an intrinsic "stickiness" of these subunits in the GST assay. On the other hand, the two-hybrid system selected only one or, at most, two members of pol subunit families in each screen but may miss some interactions. Both methods thus provide complementary views, but exhaustive two-hybrid screening with a genomic library often has the additional advantage of mapping precisely the interaction domains of the prey proteins.

Our data also can be compared with the outcome of gene-dosage suppression of thermosensitive mutations (refs. 17 and 35–39; J.-F. B., L. R., and P. T., unpublished results). As shown in Fig. 3, these two sets of data are often, but not always, convergent, which is hardly surprising as they are based on different principles. The two-hybrid screens show that two polypeptides are able to form heterodimeric associations in a reasonably physiological context, and thus may be direct physical partners in a multisubunit complex, whereas dosage-dependent suppression presumably corrects assembly or stability defects by a mass action effect, which needs not always imply a direct protein contact (see ref. 40 for a discussion of that point). For example, no two-hybrid interaction was observed by using the whole C160 subunit and any other protein. However, a thermosensitive mutation in C31 can be suppressed by C160 overexpression and a mutant form of C160 releases C31, C34, and C82 in cell-free extracts (Fig. 3), indicating that these three subunits are tethered to the enzyme via an interaction with C160 (30). These observations are in agreement with our two-hybrid data suggesting that C31, C34 and C82 are physically associated (16) as in the case of the human pol III (31).

Photoaffinity probing of the pol III initiation and elongation complexes (10, 12, 41) showed that, of nine subunits that could be crosslinked to DNA, C34 was located the farthest upstream on DNA, in agreement with its role in the interaction with the preinitiation complex (20). Moreover, both C82 and C31 are located close to C34 in agreement with their interaction with this subunit (12, 41, 42), whereas the two large subunits show crosslinking over almost the entire length of the complexes. Interestingly, photoaffinity probing of pol III transcription initiation complexes in crude extracts has revealed the photocrosslink of a polypeptide of about 40 kDa, which might correspond to C37 even though the crosslink was sensitive to heparin (41). Finally, C53 and ABC27 polypeptides were localized toward the 3′ end of the initiation complexes (12, 41).

An important outcome of the present study is the mapping of interaction domains between the two large subunits of pol I and III and eight small subunits (AC40, ABC27, AC19, ABC14.5, A12.2, C11, ABC10α, and ABC10β). ABC14.5 interacts with the loosely conserved domain **e** of the largest subunits whereas ABC27 interacts with an 80-aa sequence that overlaps domain **h**. This latter domain is located close to the end of the sequences of the large subunits (except for pol II where it is followed by the carboxyl-terminal domain) and is best conserved in archaebacteria that have an ABC27 homologue (26, 43). A conserved interaction pattern with the second large subunits also exists in the case of the homologous subunits C11 and A12.2, themselves related to the B12.6 (Rpb9) subunits of pol II (7). Our results show that C11 and A12.2 interact with the conserved and homologous N-terminal domains of C128 and A135, respectively. Interestingly, these small proteins also bear similarity to the TFIIS pol II transcription elongation factor. Recently, B12.6 and C11 were implicated in transitions between the elongation mode and the RNA cleavage mode of transcription (7, 44), suggesting that A135 and C128 N-terminal domains may themselves be critical for transcription elongation.

Intriguingly, the C53 pol III-specific subunit contacted TFIIIC. This contact is clearly not required for transcription initiation *in vitro* because a TFIIIB·DNA complex was shown to be sufficient to direct multiple rounds of transcription of naked DNA by pol III (45). However, in support of the idea of an interaction between the enzyme and its factor, a human TFIIIC-containing pol III holoenzyme recently was described (46). The role of the C53·τ131 contact is not known, but TFIIIC might contribute to the recruitment and correct positioning of pol III. Alternatively, the interaction of C53 with

the TFIIIC assembling subunit of TFIIIC might disrupt TFIIIB·TFIIIC contacts and favor TFIIIC displacement (47).

Unexpectedly, both the AC19 and the B12.5 (Rpb11) screens yielded clones encoding C-terminal fragments of the B44 pol II subunit. However, the D subunit of pol from the archaebacterium *Methanococcus jannaschii* (homologous to AC40 and B44) is able to interact with yeasts B12.5 and AC19 in the two-hybrid system (48), suggesting that the interaction surface is evolutionarily conserved. It is likely that in the pol I and pol III enzymes additional interactions are required to discriminate between AC40·AC19 and B44·AC19 heterodimers and avoid the incorporation of the latter in pol I or III.

Fig. 3 summarizes our data on subunit interactions within the pol III initiation complex, based on two-hybrid interactions and dosage-dependent suppression data (this work and refs. 16 and 17). This model incorporates data related to common or conserved subunits that are shared between pol III and the other two polymerases, and thus also pertains to the spatial organization of the three enzymes. Our interaction studies thus could be used to locate domains of subunits on three-dimensional models of pol I or pol II (2, 6, 7). For example, we propose that ABC27 is located close to the pol II carboxyl-terminal domain because it interacts with the C terminus of the large subunit (6). Similarly, part of the C-terminal third of A135 must be located in the apical region of pol I because it interacts with the AC40 and AC19 subunits, which were found to colocalize on the three-dimensional structure of yeast pol I (2). Finally, the N terminus of the second largest subunit should be located in the pols' thumb because A12.2 is located in that feature of pol I and because both C11 and A12.2 interact with that part of the second large subunit (7).

In conclusion, we have mapped many interactions between pol components, allowing a better understanding of these complex enzymes. The knowledge of the interaction domains will help address the question of the role of these interactions in eukaryotic pol and function.

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