Inter- and Intrasubunit Interactions during the Formation of RNA Polymerase Assembly Intermediate*

We used yeast two-hybrid and in vitro co-immobilization assays to study the interaction between the Escherichia coli RNA polymerase (RNAP) α and β subunits during the formation of αβ, a physiological RNAP assembly intermediate. We show that a 430-amino acid-long fragment containing β conserved segments F, G, H, and a short part of segment I forms a minimal domain capable of specific interaction with α. The α-interacting domain is held together by protein-protein interactions between β segments F and I. Residues in catalytically important β segments H and I directly participate in α binding; substitutions of strictly conserved segment II Asp1084 and segment I Gly1315 abolish αβ formation in vitro and are lethal in vivo. The importance of these β amino acids in α binding is fully supported by the structural model of the Thermus aquaticus RNAP core enzyme. We also demonstrate that determinants of RNAP assembly are conserved, and that a homologue of β Asp1084 in A135, the β-like subunit of yeast RNAP I, is responsible for interaction with AC40, the largest α-like subunit. However, the A135-AC40 interaction is weak compared with the E. coli α-β interaction, and A135 mutation that abolishes the interaction is phenotypically silent. The results suggest that in eukaryotes additional RNAP subunits orchestrate the enzyme assembly by stabilizing weak, but specific interactions of core subunits.

Cellular RNA polymerases (RNAPs)¹ are large, multisubunit enzymes. A typical prokaryotic RNAP core contains 5 polypeptides with a total molecular mass of ~400 kDa. Core RNAP from eukaryotes and archaea contain 10–14 subunits with a total molecular mass in excess of 500 kDa. Sequence alignments of RNAP subunits reveal extensive similarities; each of the two largest RNAP subunits, which are the most evolutionarily conserved, contains 8–9 colinear segments with many invariant amino acids (1, 2). RNAPs from different sources are also homologous structurally; low resolution (16–35 Å) three-dimensional models of Escherichia coli, and RNAP II and RNAP I from yeast obtained by means of electron crystallography reveal significant similarities (3–5).

Evolutionarily conserved subunit segments probably form distinct functional domains common to all RNAPs. Genetic data support this notion; mutational changes of conserved residues selectively destroy distinct partial functions of the enzyme (e.g. the transition from abortive initiation to productive elongation (Ref. 6), or phosphodiester bond synthesis (Ref. 7)), but leave other functions unperturbed. However, isolated subunits themselves do not possess any of the partial functions of the whole enzyme (8). Therefore, RNAP functional sites are either formed allosterically upon the enzyme assembly, or are located at subunit interfaces. Thus, understanding inter- and intrasubunit interactions should provide insights in RNAP mechanism and regulation.

E. coli RNAP assembles in vivo and in vitro according to the following scheme: αααααββββ → ααααββββ → αααββββ → ααββββ → αββββββββ (9). The αβi intermediate appears to be evolutionary conserved, and an αββi-like RNAP II subassembly was isolated from yeasts (10, 11). Further, mutations in E. coli α, and in its yeast RNAP II counterpart, Rpb3, that affect the αβ formation in their respective systems occur in homologous positions (12, 13).

The β and β′ homologues are naturally fragmented in some archaea and cyanobacteria (14, 15). The assembly pathway should be more complex in organisms with split β, β′ homologues, but this has not been investigated. Our own work with E. coli defined four separable domains in β and three in β′ (16, 17), that correspond to natural fragmentation sites in some of the archaea and cyanobacteria. The ability to generate functional E. coli RNAP using subunit domains greatly aided the study of RNAP assembly and biochemical functions of assembly intermediates. A combination of fragmented RNAP reconstitution, limited proteolysis, and protein-protein co-immobilization assays was used to demonstrate that determinants of specific α binding reside in the C-terminal assembly-competent structural module of β, containing amino acids 907–1342 (conserved segments H and I; Ref. 18). However, similar analysis performed by the Ishihama group (19) suggested that the primary determinants of α binding include conserved segments F and G, corresponding to E. coli β amino acids 800–900.

The discrepancy between the two sets of data illustrates problems associated with in vitro coimmobilization assays, which can be artifact-prone. Thus, alternative approaches are needed to characterize the α-β interaction in molecular detail. Here, we used the method of yeast two-hybrid analysis to analyze the interaction of the α and β subunits during αβ formation. Our results support the conclusion of Wang et al. (18) and directly implicate two strictly conserved amino acids in β segments H and I in interactions with α.

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¹ The abbreviations used are: RNAP, RNA polymerase; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; NTA, nitritoltriacetic acid; Rif, rifampicin; NTD, N-terminal domain; Gal4-DB, GAL4 DNA binding domain; Gal4-AD, GAL4 activation domain.

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MATERIALS AND METHODS

Proteins and Plasmids—Gal4-based two-hybrid plasmids pPC97 (bait plasmid) and pPC86 (prey plasmid) were described in Ref. 20.

α Derivatives—The two-hybrid plasmids expressing α NTD (rpoA codons 1–235) were constructed by PCR cloning using E. coli expression plasmids containing either wild-type rpoA or rpoA harboring the B45A mutation as templates (18). E. coli plasmid expressing His6-αNTD, and conditions for protein expression and purification are described in Ref. 21.

β Derivatives—The two-hybrid plasmids expressing various fragments of β used in this work were constructed by PCR cloning using E. coli pMK52 expression plasmid (22) as a template. Site-specific PCR mutagenesis and standard reconstituting steps were used to introduce point mutations in yeast two-hybrid of E. coli expression plasmids. Full-length β and β′ were used in vitro RNAP assembly studies were obtained from expression plasmids pMK52 and pT7/β′ (23), respectively. Overexpression was induced with 1 mM isopropyl-β-D-thiogalactoside at 30 °C for 3 h. The proteins in inclusion bodies were then prepared according to Ref. 21.

Yeast RNAP Subunit Derivatives—The two-hybrid plasmids expressing various fragments of yeast RNAP subunits were constructed using appropriate primers and high fidelity PCR with yeast genomic DNA as a template. Plasmid pNOY302, which harbors the entire A135 gene, was provided by M. Nomura and is described in Ref. 24.

Yeast Strains and Techniques—Yeast were transformed using standard lithium acetate procedure, and transformants were selected on appropriate drop-out media. For two-hybrid assay, we used the LNY327 test strain provided by L. Neigeborn. This strain is identical to the PCY2 strain of Chevrai and Nathans (20). The interaction between two-hybrid constructs was scored using filter assays, or quantitative colorimetric assay using ortho-nitrophenyl-β-galactoside as a substrate (25). The following formula was used to calculate β-galactosidase production: 

$$
\text{β-galactosidase activity} = \frac{10,000 \times (OD_{420} - 1.75 \times OD_{550} \times OD_{660} \times \tau)}{OD_{240} \times OD_{560}}
$$

where OD420, OD550, and OD660 are optical density of the reaction at 420, 550, and 600 nm, respectively, at time t (min) of the reaction.

Yeast tester strain NOY302-1a was provided by M. Nomura and has been described previously (26).

Reconstitution of αβ Complexes and RNAP—Purified His6-αNTD was mixed with β or its derivatives (from washed inclusion bodies) at a molar ratio of 1:1 and a total protein concentration of not more than 0.5 mg/ml in reconstitution buffer (40 mM Tris-HCl, pH 7.9, 100 mM NaCl, 10 mM EDTA, 20 mM MgCl2, 10 μM ZnCl2, 20% (v/v) glycerol, 2 mM β-mercaptoethanol, 6.3 guanidine HCl) and dialyzed overnight at 4 °C against 2 × 2 liters of the same buffer without guanidine HCl. For RNAP reconstitution, proteins were combined at a molar ratio of α:β′ of 1:1:2. At these conditions, unaseable β′ precipitated during dialysis and was removed by low speed centrifugation.

Ni2+-NTA-Agarose Co-immobilization Binding Assay—RNAP complexes were mixed with pre-equilibrated Ni2+-NTA-agarose beads (Qiagen) in 50 μl of reconstitution buffer and incubated for 10 min at room temperature with gentle mixing. The beads were pelleted by centrifugation and washed twice with 500 μl of buffer with 10 mM imidazole. The protein samples were then eluted from the beads with buffer containing 100 mM imidazole and analyzed by SDS-PAGE.

In Vitro Transcription—10 μl of RNAP reconstitution reaction was incubated for 15 min at 37 °C in the presence of 1 μg of recombinant αβ subunit (23), and 50 ng of the T7A1 promoter-containing DNA fragment (22), 0.5 mM CapA, with or without 25 μg/ml rifampicin. Reactions were initiated by the addition of NTP (final concentration 25 μM ATP, CTP, and GTP, 1 μM [α-32P]UTP (1000 Ci/mmol). Reactions proceeded for 15 min at 37 °C, and were terminated by the addition of urea containing loading buffer. Reaction products were resolved by denaturing 20% PAGE and revealed by autoradiography.

RESULTS

The Interaction between E. coli RNAP α and β Can Be Detected in Yeast Two-hybrid System—For our research, it was critical to establish that the interaction between β and the dimeric α subunit could be studied by two-hybrid approach, and that mutations that decrease this strong interaction can be detected in yeast. As an initial test, we used the N-terminal domain of α (NTD, amino acids 1–235) and the β subunit fragment containing amino acids 711–1246, which specifically interact with each other in vitro (18). α-NTD was fused to the GAL4 DNA binding domain (Gal4-DB) of the bait plasmid pPC97, and β711-1246 was fused to the GAL4 activation domain (Gal4-AD) of the prey plasmid pPC86 (20). High levels of β-galactosidase were observed when both plasmids were present (intense blue color developed in less than 30 min in a standard filter assay; 440 Miller units of β-galactosidase activity in a quantitative liquid assay, see “Materials and Methods”); no activity was observed when either fusion plasmid was used separately, as expected (cells remained white after overnight incubation on a filter, less than 1 Miller unit of β-galactosidase activity). Importantly, when pPC97α1-235 carrying a mutation that changes α Arg45 for Ala and weakens the interaction with β (12), was used, no activity was detected even in the presence of pPC86β711-1246 (white color, less than 1 Miller unit of β-galactosidase activity, data not shown). Thus, we can detect the α-β interaction and amino acid changes that affect this interaction using the two-hybrid system. This result sets the stage for more detailed analyses of RNAP assembly described below.

Defining the Minimal Fragment of β Capable of Interaction with α—Our starting β two-hybrid construct, pPC86β711-1246, contained 535 rpoB codons and corresponded exactly to the smallest β fragment that specifically interacted with α in vitro (18). The β711-1246 fragment spans four universally conserved β segments, F, G, H, and I, and part of I. To determine the smallest β fragment capable of interacting with α, we engineered pPC86β800-1246 derivatives with deletions at either the beginning or the end of the β moiety, and tested these new plasmids for their ability to elicit β-galactosidase production in yeast cells harboring pPC97α1-235. The results are schematically presented in Fig. 1.

As can be seen, β800-1246 hybrid interacted with the α hybrid. However, further deletion in segment F, β800-1231, abolished the interaction. At the C-terminal side, removal of β amino acids 1231–1246 had no effect on interaction with α. However, the removal of additional 6 β amino acids (1226–1231) abolished the interaction. The results suggest that β amino acids 711–800, which contain 80 non-conserved amino acids, and 10 amino acids from conserved segment F, and β amino acids 1231–1246, which contain most of β conserved segment I, including residue His1237, which forms the 5′-face of the catalytic center (27), are not necessary for specific interaction with α. We note, however, that these regions of β may still contribute to the overall strength of the binding, since the two-hybrid interaction may be saturated and insensitive to minor changes in the strength of binding.

The two-hybrid construct containing β amino acids 800–1231 was the smallest construct that interacted with α strongly (520 Miller units) and specifically (0.5 Miller units when used with αR45A two-hybrid construct) (Fig. 1). Shorter constructs, constructed by site-directed mutagenesis, β800–1231, β800–1221, and β800–1225, failed to interact with α hybrid (≤1 Miller unit).

We also attempted to further narrow the interacting domain by performing nested Bal31 deletion mutagenesis at either the 5′ or the 3′ ends of the rpoB portion of pPC86β800–1231, transforming mutated plasmids in yeast cells harboring pPC97α, and selecting interacting (Lac−) colonies. The DNA sequence of several interacting β plasmids was determined, and in all cases Bal31 deletions extended into the vector part of pPC86β800–1231, while rpoB codons 800 and 1231 were retained (data not shown). We conclude that β800–1231 is the minimal β fragment capable of α binding.

The minimal interacting fragment of β, β800–1231, contains dispensable region II (β amino acids 907–1050) that is missing in some bacterial β subunits and in homologues from eukaryotes and archaebacteria (28). This region tolerates an artificial split at around position 950 (16), and is highly susceptible to...
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Fig. 1. Determining the minimal β fragment capable of interacting with α in the two-hybrid screen. The horizontal structure at the top represents the primary sequence of β. Evolutionarily conserved regions are shaded gray and labeled E-I according to Ref. 2. The open boxes represent regions containing large deletions found in β homologues from Gram-positive bacteria and chloroplasts. Dispensable regions are represented as bars above the primary structure. The locations of Lys<sup>1065</sup> and His<sup>1237</sup>, which cross-link to initiating nucleotide analogs (27), are indicated (bars). The vertical structure is the results of in vitro trypsin cleavage of the β fragment containing evolutionary conserved glycines for aspartic residues in β modules, consistent with the idea that segment F and I amino acids contribute to α binding indirectly.

Point Mutations in β Segment H and I Affect α Binding in Vivo and in Vitro—In α, substitution of the strictly conserved Arg<sup>22</sup> interferes with the β binding (12). We hypothesized that Arg<sup>22</sup> interacts with a negatively charged, conserved amino acid in β, and that this interaction is responsible for the αβ formation. Accordingly, we inspected the E. coli β fragment between amino acids 800 and 1231 to see if such amino acids could be found. Segment G contains no conserved negatively charged amino acids; segment F contains one such residue, Glu<sup>813</sup>. This position was previously mutated to an Arg (29); the resulting RNAP had a catalytic defect but had no assembly defect, and therefore we did not study this position further. As can be seen from the alignment presented in Fig. 3A, segment H contains 4 negatively charged amino acids, Asp<sup>1064</sup>, Asp<sup>1085</sup>, Glu<sup>1114</sup>, and Glu<sup>1114</sup>, which are strictly conserved and thus fulfill our criteria for potential candidates involved in α binding. Residue Asp<sup>1064</sup> was mutated previously in the course of a mutational study of the RNAP initiating site (30). The results of that study revealed that β harboring the DI064A mutation was assembly-proficient, but the resulting RNAP was catalytically defective. Based on these results, position 1064 was excluded from our analysis. Segment I contains no conserved negatively charged amino acids. However, there are three Gly residues, at positions 1215, 1218, and 1228, which are strictly conserved. Previously, we had demonstrated that substitutions of evolutionarily conserved glycines for aspartic residues in the β subunit result in assembly defects (31). Therefore, in addition to the three site-specific mutations changing two aspartic and one glutamate from segment H for alanines, we also substituted each of the three segment I glycines for aspartates. The mutations were engineered in pPC86β<sup>Δ1203</sup> background, and their effect on α binding in two hybrid system was studied. The results are shown above the alignment on Fig. 3A. Of the
six mutations tested, only two, D1084A and G1215D, completely abolished the interaction as judged by both the filter and the liquid β-galactosidase assays. G1218D showed weak, but clearly positive, interaction. The remaining mutations had no effect on α binding.

To confirm the α binding defects directly, we reconstituted all mutations into the pMKSe2(S531F) β-overproducing plasmid (22). The β subunit expressed from pMKSe2(S531F) contains a rifampicin (Rif) resistance mutation changing Ser531 to Phe. Therefore, Rif-sensitive host cells transformed with pMKSe2(S531F) grow on medium containing Rif. In contrast, cells transformed with pMKSe2(S531F) harboring the engineered segment H and I mutations did not form colonies in the presence of Rif, but were unaffected for growth in its absence. Thus, the rpoB mutations behaved as recessive lethals, a phenotype expected of assembly mutations.

The binding defect of β mutants was also showed directly, by studying their ability to co-immobilize on Ni²⁺-NTA-agarose through protein-protein interactions with hexahistidine-tagged αNTD (Fig. 3B). In this experiment, the wild-type or mutant β subunits were prepared from inclusion bodies and combined with recombinant αNTD in the presence of high concentration of denaturing agent guanidine HCl. The denaturant was dialyzed away at conditions favoring RNAP assembly, and reactions were allowed to bind to Ni²⁺-NTA-agarose beads. The beads were washed, and the bound protein was eluted with a buffer containing high concentration of imidazole. As can be seen, the wild-type β was efficiently immobilized on the sorbent in the presence of tagged αNTD (Fig. 3B, lane 4), as expected. In contrast, most of β harboring the D1084A and G1215D substitutions was found in the flow-through (lanes 6 and 10) and did not associate with αNTD (lanes 8 and 12). G1218D also caused a defect in αNTD binding, but, in agreement with the two-hybrid results, the extent of this defect was weaker than those of either D1084A or G1215D (compare lane 20 with lanes 8 and 12). Similar analysis revealed that in agreement with the two-hybrid result substitutions at positions 1095, 1114, and 1228 had no effect in the co-immobilization assay (Fig. 3B, lane 24; and data not shown). We conclude that β positions 1084, 1215, and, to a lesser degree, 1218 are involved in α binding, and that the lethal in vitro phenotypes exhibited by the rpoB genes harboring substitutions in these positions are likely explained by the defect in the αβ interaction. In contrast, the lethal phenotype caused by substitutions at rpoB positions 1095, 1114, and 1228 must be due to alterations of some other essential RNAP function(s).

In principle, two classes of mutations can abolish protein-protein interaction. The mutations of the first class distort the native structure, perhaps through an allosteric mechanism, and thus prevent the interaction. The second, most interesting class, is true interaction mutations, which affect favorable interactions that bring the two proteins together. We investigated the ability of the β subunit harboring the D1084A and G1215D mutations to assemble into active RNAP in vitro in the presence of the wild-type β’ and hexahistidine-tagged αNTD. The assembly reactions were examined by Ni²⁺-NTA coimmobilization (Fig. 3C). The addition of β’ corrected the αNTD-binding defect caused by the D1084A mutation, and RNAP core enzyme was formed in good yield (Fig. 3C, lane 8). A steady-state in vitro transcription experiment revealed transcription activity in the RNAP assembly reactions containing β with D1084A substitution (Fig. 3D, lane 3). This transcription was rifampicin-resistant (lane 4), proving that the activity was due to the mutant RNAP, which also carries the S531F Rif resistance mutation, and not due to contaminating wild-type RNAP from the host, which is rifampicin-sensitive (see lanes 5 and 6). This result implies that the D1084A mutation does not grossly distort β structure and may be directly involved in interactions with α. In contrast, no assembled RNAP was detected in the in vitro RNAP assembly reaction containing His-tagged α, β’, and β carrying the G1215D mutation (Fig. 3C, lane 12), and there was no transcription activity in G1215D assembly reactions (Fig. 3D, lanes 7 and 8). Thus, Gly1215 appears to play a structural role, and its involvement in α binding may be indirect.

Evolutionary Conservation of the β- and α-like RNAP Subunit Interactions—In yeast, two α-like subunits, AC40 and AC19, form a heterodimer that is functionally equivalent to the α homodimer in prokaryotes (32). Interestingly, AC40 and AC19 are shared by RNAP I and RNAP III. Thus, interaction of the RNAP I α-like subunit, A135, and its RNAP III homolog, C128, with the AC40/AC19 heterodimer could determine the relative amount of RNAP I and RNAP III in the cell. We wanted to know (i) whether C-terminal A135 and C128 fragments contained determinants for interaction with the α-like subunits, as would be expected from our E. coli results, and (ii) whether C128 and A135 interact with the same α-like subunit in the heterodimer. Two-hybrid plasmids expressing the N- and C-terminal halves of A135 and C128 as defined by the archaeal split were constructed. The two-hybrid plasmids were then tested for their ability to elicit α-galactosidase production with complementary plasmids expressing either AC19 or AC40 hybrids. As control, we used the AC40 and AC19 pair, which had
previously been shown to interact in two-hybrid assay. The results can be summarized as follows. First, as expected, AC40 fused to Gal4-DB showed strong positive interaction with AC19-Gal4-AD (120 Miller units). Second, the N-terminal fragments of the β-like subunits, containing conserved segments A, B, C, D, and E (residues 1–728 and 1–717 in C128 and A135, respectively) did not interact with the α-like subunits. Third, C-terminal fragment of C128 (residues 729–1149 conserved segments F, G, H, and I) also did not interact with either AC19 or AC40 hybrids, and thus no conclusions about the formation of RNAP III α2β-like structure could be made. Finally, weak (13 Miller units) interaction between the C-terminal fragment of A135 (residues 717–1203) with the AC19 hybrid, but not with the AC40 hybrid was detected.

Since the α-like subunits AC19 and AC40 heterodimerize with high efficiency (32, see also above), the observed interaction between the A135 β subunit and AC19 two-hybrid constructs is likely due to A135 Gal4-AD hybrid interaction with AC40/AC19 Gal-AD heterodimer. We constructed an AC19 two-hybrid plasmid, which contained a double-alanine substitution at AC19 positions 78, and 79, corresponding to E. coli positions 44 and 45, and should have abolished A135 binding to AC19. The resulting construct was unaffected in A135 interaction (15 Miller units), suggesting that conserved residues in the α-motif...
The results of growth after a 62-h incubation at 30 °C are presented.

Intrasubunit Interaction of between Segment F and I Residues Is Important for a Binding—Our results demonstrate that the smallest fragment of E. coli β capable of specific α binding spans amino acids 800–1231. The structural context of the corresponding fragment of Taq β is shown in Fig. 5 (A–C). As can be seen, the fragment has an elongated shape and consists of two domains. One domain is formed entirely by conserved segment G and contains the so-called “flexible flap” element, which is thought to interact with RNA at hairpin-induced pause sites (35), and harbors the site of trypsin attack (28). The second domain contains the entire conserved segment H as well as segments F, and I, which are far away from each other in the primary sequence. The second domain is involved in protein–protein interactions with one α monomer, α1. The second α monomer, α2, does not interact with β and instead contacts β′.

The α-binding domain is held together by a 6-strand antiparallel β-sheet. Conserved segments F, H, and I contribute to this β sheet, and stretches of amino acids corresponding to E. coli 800–812 and 1226–1231 each form interacting strands (Fig. 5D). We registered this intrasubunit interaction as a strong β-galactosidase production when hybrids containing E. coli β800–907 and β951–1231 were combined. Removing either segment F or segment I amino acids disrupts the structure of the entire α-binding domain, and as a consequence abolishes α binding.

Segment H and I Amino Acids Participate in a Binding.—The prototypical E. coli mutation that decreases the avidity of α binding to β changes a Arg45 which is evolutionarily conserved (12). We hypothesized that, in β, the likely counterpart of α Arg45 is negatively charged and evolutionarily conserved. Out of three possible candidates, all of which were located in segment H, only one, Asp1084, when mutated affected α binding both in two-hybrid assay, and in vitro co-immobilization experiments. In addition, substitution of segment I Gly1215 also abolished α binding.

The structure reveals that Taq Asp857, which is homologous to E. coli β Asp1084, is indeed in direct contact with Arg45 (E. coli Arg45) in α1 (Fig. 5E). Taq Gly977, which is homologous to E. coli β Gly1215, is located at the tip of a loop formed by segment I and buttresses Asp857 (E. coli Asp1084) to position it such that it can correctly interact with Arg42 of α1. The G1215D substitution that we engineered probably causes unfavorable electrostatic interaction with Asp1084 and may significantly alter the conformation of the α-binding domain. In agreement with this idea, G1215D abolished the β′ entry in the complex. In contrast, RNAP harboring β D1084A mutation assembled efficiently and was active. Thus, β′ can stabilize the αβ subassembly, presumably through independent protein–protein interactions with the second α monomer, α2, and β. Of the remaining four mutations that we engineered, one (G1218D) substantially decreased α binding, while others had no effect. Taq Gly870 (E. coli Gly1218) is in the same loop as Taq Gly977 (E. coli β Gly1215); it makes no contacts with α and is at least 10 Å away from Arg42 (E. coli Arg45). Thus, G1218D substitution inhibits α-binding indirectly, probably by affecting the relative position of the stabilizing Gly1215. The importance of precise positioning of α Arg45 and β Asp1084 for αβ formation is underscored by the fact that no interaction was detected when a pair of mutants containing an Asp in place of Arg45 and an Arg instead of Asp1084 was tested (data not shown).
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The α-binding domain of β also contains two strictly conserved residues that are implicated in catalysis, and that are exposed into the DNA-binding channel of the enzyme, close to the catalytic Mg ion. In segment H, Taq Lys838 (Lys1066 in E. coli) contacts the α-phosphate of the initiating purine nucleotide (6); in segment F Taq Glu885 (Gly813 in E. coli) is probably required for proper interaction with incoming NTP (29). Binding of α thus stabilizes the active center conformation. In agreement with this idea, we had found that in the absence of β’, α is necessary to induce specific binding of initiating purine nucleotide by the β subunit.2

Yeast RNAP Assembly—Our studies of yeast RNAP subunit interactions reveal several important points. First, as expected, the determinants of α binding reside in the C-terminal portion of A135, the β-like subunit of RNAP I, and Asp935, which is homologous to E. coli β Asp1084, is required for this interaction. This result is in agreement with recent RNAP interaction mapping by Flores et al. (36). These authors reported that A135 segment 678–1055, containing conserved segments E, F, G, H, and Ic (corresponding to E. coli β positions 642–1253), bound both AC19 and AC40 in two-hybrid assay. Our interacting fragment of A135 contained conserved segments F, G, H, and entire I (amino acids 716–1201), and corresponded to E. coli β residues 681–1342. Based on mutational analysis, A135 appears to interact with the larger α-like subunit, AC40, and not with the smaller AC19. This is consistent with structural analysis of yeast RNAP II by the Kornberg group (34), who observed that the largest α-like subunit, Rpb3, contacts the β-like Rpb2. Our data indicate that, in yeast, the interaction between the β-like and the α-like subunits of RNAP I is much weaker than the corresponding interaction in E. coli, and A135 mutations that abolish this interaction are viable. The corresponding interaction may be even weaker in case of RNAP III. We and others (38) were unable to detect any interaction between RNAP III β-like subunit C128 and AC40/AC19 using two-hybrid assay. Additional RNAP subunits may strengthen the β-like complex in eukaryotes. Indeed, the AC40/AC19 heterodimer was shown to bind ABC10 (Rpb10) shared RNAP subunit (32). More recently, crystallographic analysis revealed that ABC10β and ABC10α (Rpb12) bind to the larger α-like subunit Rpb3 in yeast RNAP II and anchor it on the β-like Rpb2 (34). An alternative possibility would be that the eukaryotic αβ-like complex is stabilized by the entry of the largest, β-like subunit in the complex, similar to the situation observed with E. coli β D1084A mutation. This latter scenario envisions that the relative amount of RNAP I and III in the cell is chiefly determined by the joint availability of catalytic β and β'-like

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subunits, which are specific, and not by the shared α-like subunits.

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