The Rpb9 Subunit of RNA Polymerase II Binds Transcription Factor TFIIE and Interferes with the SAGA and Elongator Histone Acetyltransferases*

Received for publication, July 30, 2001; in revised form, January 4, 2002
Published, JBC Papers in Press, January 4, 2002, DOI 10.1074/jbc.M107207200

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Rpb9 is a small subunit of yeast RNA polymerase II participating in elongation and formed of two conserved zinc domains. rpb9 mutants are viable, with a strong sensitivity to nucleotide-depleting drugs. Deleting the C-terminal domain down to the first 57 amino acids has no detectable growth defect. Thus, the critical part of Rpb9 is limited to a N-terminal half that contacts the lobe of the second largest subunit (Rpb2) and forms a β-addition motif with the “jaw” of the largest subunit (Rpb1). Rpb9 has homology to the TFIIS elongation factor, but mutants inactivated for both proteins are indistinguishable from rpb9 single mutants. In contrast, rpb9 mutants are lethal in cells lacking the histone acetyltransferase activity of the RNA polymerase II Elongator and SAGA factors. In a two-hybrid test, Rpb9 physically interacts with Tfa1, the largest subunit of TFIIE. The interacting fragment, comprising amino acids 62-164 of Tfa1, belongs to a conserved zinc motif. Tfa1 is immunoprecipitated by RNA polymerase II. This co-purification is strongly reduced in rpb9-Δ, suggesting that Rpb9 contributes to the recruitment of TFIIE on RNA polymerase II.

The recent determination of the bacterial RNA polymerase (1) and yeast RNA polymerase II (Pol II) (2) core structures has opened a new chapter in transcription studies. The remarkably similar organization of these two enzymes leaves no doubt as to the strong mechanistic conservation of the transcription process. This similarity was anticipated from the sequence homology existing between the ββααββαα dimer bacterial core enzyme and 5 of the 10 core subunits of Pol II. Rpb1 and Rpb2 are homologues of ββααββαα, the Rpb3/Rpb11 heterodimer corresponding to the bacterial α2 dimer, and αβ is distantely related to Rpb6 (9). On the other hand, Pol II contains five small subunits (Rpb5, Rpb8, Rpb9, Rpb10, Rpb12) not found in the bacterial enzyme. These subunits also belong to the core structure of Pol I and Pol III or are related to it, in the case of Rpb9. Except for Rpb8, they are also akin to archaeal polypeptides (4, 5).

The present study deals with the Rpb9 subunit, belonging to a conserved family of eukaryotic and archael zinc-binding polypeptides that also includes the yeast Pol I (Rpa12 (6)) and Pol III (Rpc11 (7)) subunits and the TFIIS elongation factor (8–10) encoded by PPR2 in Saccharomyces cerevisiae (11, 12). There is ample evidence that Rpb9 (9), Rpc11 (7), and TFIIS (9, 13) control transcription elongation by activating the RNA cleavage activity inherent to all RNA polymerases. The fact that yeast ppr2 (14), rpb9 (15), and rpa12 (16) mutants are strongly sensitive to nucleotide-depleting drugs is also consistent with a major elongation defect. Rpb9 is highly conserved in evolution, and the yeast subunit can be replaced in vivo by its human counterpart (17). However, animal mutants (Drosophila melanogaster) are lethal (18), whereas yeast null mutants have only a limited growth defect (19).

Rpa12, Rpb9, andRpc11 have a related organization in Pol I, Pol II, and Pol III, respectively. These three subunits are essentially made of two zinc-binding domains. Moreover, Rpa12 and Rpb9 hold equivalent positions in the spatial structure of Pol I and Pol II, at the edge of their DNA channels in the “upper” side, which is occupied mainly by the second largest subunit (2, 7). It was recently shown that the entire C-terminal half of Rpa12 can be removed with no growth defect and without impairing its assembly into Pol I (16, 20). We therefore decided to examine more closely the growth properties of the rpb9 mutant altered in its N-terminal and C-terminal zinc domains and to look for partners of that subunit with other components of the RNA polymerase II transcription complex. Our data revealed a functional connection of Rpb9 with the histone acetyltransferase activity of the Pol II Elongator and SAGA complex and a physical association between Rpb9 and TFIIE. The C-terminal zinc fold of Rpb9 is entirely dispensable in vivo, despite its high sequence conservation. These data will be discussed in the light of models currently proposed for the control of Pol II elongation (21, 22).

**EXPERIMENTAL PROCEDURES**

Strains CMYK1 (ppr2-Δ:URA3) (23), SL9-6b (rpa12-Δ:LEU2) (16), and YW9 (rpb9-Δ:HIS3) (19) have been described. YYV9 (MATα ura3-52 his3-Δ200 leu2 lys2 ade2-1 trp1-Δ63 rpb9-Δ:HIS3) and D386-9b (MATα his3-Δ200 lys2-Δ201 rpb9-Δ:HIS3 trp1-Δ63) were obtained by a WY9 × YPH500 metiotic cross. OGI0-4e is a gcn5-Δ:HIS3 mutant, constructed in YPH500 (MATα ura3-52 his3-Δ200 leu2 lys2 ade2-1 trp1-Δ63) (24) by replacing the GCN5 coding sequence with a HIS3 cassette. YYV50 (rpb3-3:HA) is a YPH500 mutant that encodes a

* The collaboration between our two laboratories was supported by the Commissariat Général aux Relations Internationales (Belgique) and by INSERM (France). The costs of publication of this article were defrayed in part by the payment of page charges. This article must be discussed in the light of models currently proposed for the control of Pol II elongation (21, 22).

‡ Supported by the Belgian Fonds pour la Formation à la Recherche dans l’Industrie et dans l’Agriculture and by a Training and Mobility Grant from the European Union.

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¶ Published, JBC Papers in Press, January 4, 2002, DOI 10.1074/jbc.M107207200
mutant form of the Pol II subunit Rpb3 tagged with a C-terminal hemagglutinin epitope (see Ref. 25); a metiotic cross with YV99 yielded the YVY51 (rpb3::3HA rpb9·Δ) segregant. These strains were used to immunopurify Pol II as described previously (16). Western blots were revealed with polyclonal antibodies against Rpb2 (26) and with mouse monoclonal anti-HA and anti-Myc (Babco). The fta1 alleles were described previously (27). All other yeast strains were from Euroscarf.

They are full deletions generated by inserting the KAN-M4 cassette in strain BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 uro3Δ3). Yeast growth media (16), two-hybrid assays (28), and mutagenesis methods (29) were as described previously.

Plasmids are listed in Table I. pGEN-13MYC was constructed by directional cloning of the 552 nt BamHI-EcoRI fragment from pFA6–12MYC (25) in the yeast expression vector pGEN (30). pVV101 is a pGEN-13MYC derivative obtained by PCR cloning of the TFA1 coding sequence at the BamHI site upstream of the 13MYC epitope. Plasmids bearing full-length or partially deleted RPB9 alleles (pVV25-pVV111) were generated by PCR amplification of the appropriate oligonucleotides, ending with the BamHI and ClaI sites, and cloned in the centromeric vector pCM185 (31). Two-hybrid plasmids were constructed as follows. pGBT9-B9 was obtained by cloning the full-length RPB9 between the BamHI and SalI sites of pGBT9 after PCR amplification. pVV49, pVV50, and pVV51 were made by cloning the corresponding C-terminal Half of Rpb9 Is Entirely Dispensable in Vivo—Rpb9 is made of three distinct domains in the spatial structure of Pol II (2). As shown in Fig. 1A, each of these domains interacts with a different region of the two large subunits (Rpb1 and Rpb2). The N-terminal zinc fold (Zn1, positions 1–39) contacts the Rpb2 lobe. The β sheet linker (β4, positions 40–52) forms a strong β-addition motif with β28 on the Rpb1 jaw. The C-terminal zinc ribbon (Zn2, positions 53–122) interacts with the Rpb2 funnel (Fig. 1A). An invariant DPTLP motif on the zinc ribbon has been reported to be critical for the binding of Rpb9 to Pol II in vitro (33).

Rpb9·Δ mutants have a partial growth defect (19) and are sensitive to nucleotide-depleting drugs (15). Fig. 2 shows that large C-terminal deletions (rpb9·Δ,157, rpb9·Δ,166, rpb9·Δ,170, and rpb9·Δ,174) are indistinguishable from wild type in terms of cell growth and drug sensitivity. The fact that these deletions always retain the β4 sheet (e.g. rpb9·Δ,157) but that a more extensive deletion (rpb9·Δ,174) eliminates the latter region behaves like a null mutant (15) underscores the importance of the critical for the binding of Rpb9 to Pol II

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In an attempt to identify physical partners of Rpb9, we therefore used a GAL4BD::Rpb9 fusion as bait in a two-hybrid screening based on a library of yeast genomic fragments (32). Our previous work on other Pol I, Pol II, and Pol III subunits (16, 28, 29) has documented the relevance of this method as a way of mapping interaction domains in the transcription machinery.

This two-hybrid approach yielded one interesting candidate that encoded an internal fragment of Tfa1, the largest subunit of the heterodimeric TFIIE transcription factor (Fig. 4A). This interaction is specific of Rpb9 because Tfa1 does not interact with Rpa12 and was never isolated when screening the same library with other RNA polymerase subunits (28, 29). The two-hybrid response initially obtained with the entire Rpb9 subunit was rather weak, but a strong signal was observed when using an N-terminal deletion removing the first 32 amino acids (GAL4BD::rpb9-33,122). The latter observation implies that the N-terminal zinc domain of Rpb9 is not required for this interaction. However, attempts to more precisely identify which part of Rpb9 is critical have not been conclusive, because neither the N-terminal zinc domain nor the C-terminal zinc ribbon alone were competent for this interaction.

Tfa1 itself is evolutionarily conserved on its N-terminal half, whereas the C-terminal half is not, and it can be deleted with only a minor growth defect (27, 40). The fragment recognized by Rpb9 (positions 62–164) corresponds to the conserved part and includes a typical zinc-binding motif. Mutations that are expected to strongly distort that motif such as tfa1-C127W are viable with a conditional growth defect (27). They also do not alter the two-hybrid response of the GAL4BD::Rpb9 fusion, indicating that the integrity of the zinc motif is not required for this interaction (Fig. 4B). Intriguingly, tfa1-C127W is strongly sensitive to mycophenolate. Moreover, it is synthetic lethal with rpb9-Δ (Fig. 4, C and D). This lethality is not a general consequence of Pol II-defective mutants, because rpb1-1 mutants (growing slowly and leading to a strong and rapid arrest at 37 °C (39)) are not affected by tfa1-C127W (data not shown).

Yeast TFIIE directly binds the Pol II core enzyme (41) and co-purifies with at least one form of Pol II holoenzyme (42). The two-hybrid interaction found here evidently suggests that TFIIE binding is mediated by Rpb9 via its Tfa1 subunit. To explore this point, we immunopurified Pol II (using a Rpb3::3HA tag) in wild-type and rpb9-Δ strains expressing an epitope-tagged form of Tfa1 (Tfa1::13MYC). Because rpb9-Δ has no effect on the subunit composition of the purified Pol II (39), differences in the co-purification pattern of Tfa1 can confidently be ascribed to Rpb9 itself. Fig. 5 shows that Tfa1::13MYC co-purifies with the immunoprecipitated Pol II, that rpb9-Δ strongly impairs this co-purification, and that the mutant enzyme nevertheless retains some Tfa1 binding. Taken together, these data strongly suggest that TFIIE binds Pol II by at least two contact points, one of which involves a direct interaction between Rpb9 and the conserved N-terminal part of Tfa1.

**DISCUSSION**

Rpb9 is formed of two zinc domains that are strongly conserved from yeast to man (17) and define two separate folds in the spatial structure of Pol II (2). In vitro, this subunit controls...
Fig. 3. Functional interaction between rpb9-Δ and other components of the transcription machinery. A, lack of synergy between rpb9-Δ and ppr2-Δ. YVV9 (rpb9-Δ) and CMKv1 (ppr2-Δ) were crossed, and 40 offspring tetrads were analyzed. This high number was necessitated by the genetic linkage of these two genes. All of the rpb9-Δ ppr2-Δ double mutants obtained had the same rpb9-Δ-like growth response. A typical growth pattern is shown for rpb9-Δ ppr2-Δ, rpb9-Δ, ppr2-Δ and wild-type (YPH500) strains streaked on SC or SC + mycophenolate at various concentrations (shown here for 1 μg/ml) and incubated for 6 days at 25 or 34 °C. B, synthetic lethality between rpb9-Δ and mutants of SAGA and Elongator complexes. YVV9 (rpb9-Δ) was crossed with Y02742 (elp3-Δ), Y03078 (sas3-Δ), Y05608 (hpa2-Δ), OG30-4c (gen5-Δ), Y04282 (ada2-Δ), Y03534 (ada3-Δ), and Y04226 (spt3-Δ). The offspring tetrads were grown on YPD for 5 to 7 days at 25 °C. The black triangles indicate the position of the double mutant spore. Synthetic phenotypes are indicated as follows: +, wild-type growth; (+), partial growth defect; −, lethality. For each cross, 20 tetrads were analyzed, and a typical tetra-type segregation is shown. C, synergy between rpa12-Δ and gen5-Δ. rpa12-Δ gen5-Δ was obtained by crossing rpa12-Δ (SL9-6b) with gen5-Δ (OG30-4c). Cells were grown on YPD at 30 °C for 7 days. Growth of a wild-type strain (YPH500) and parental rpa12-Δ (SL9-6b) and gen5-Δ (OG30-4c) mutants is shown for comparison. D, viability of gen5-Δ rpb1-1 double mutant. A gen5-Δ rpb1-1 double mutant was obtained by crossing gen5-Δ (OG30-4c) with rpb1-1 (BY260), and was streaked on YPD and incubated at 30 °C for 5 days. Growth of a wild-type strain (YPH500) and both parental single gen5-Δ and rpb1-1 mutants is shown for comparison.

The elongation defect at the level of the cleaving RNase activity (9). This elongation defect is consistent with the strong sensitivity of rpb9 null mutants to nucleotide-depleting drugs (15). However, the C-terminal zinc ribbon of Rpb9 can be entirely deleted, down to the first 57 amino acids of Rpb9, without any detectable growth defect and with no increased sensitivity to nucleotide-depleting drugs. Thus, only two elements of Rpb9 are critical for its biological activity, its N-terminal zinc fold (position 1–39), which interacts with the Rpb2 “lobe” in the Pol II spatial structure (2), and a β4 “linker” sheet forming a β-addition motif with β28 on the Rpb1 “jaw.” This supports the existence of a mobile lobe-Rpb9-jaw module, as predicted from a comparison of the two structures adopted by Pol II crystals (2). The fact that the contact between Rpb9 and the Rpb1 funnel can be disrupted with no detectable effect is surprising given the high conservation of the funnel and that it physically connects Rpb9 to the catalytic pocket of the enzyme. There is a striking parallel with the Rpa12 Pol I subunit in Schizosaccharomyces pombe (20) and in S. cerevisiae (16). In particular, a rpa12 deletion retaining the first 60 amino acids of the S. cerevisiae subunit has a wild-type growth and drug sensitivity pattern and suffices to incorporate the mutant subunit into Pol I (16). This parallel is also underscored by the common sensitivity of rpb9 and rpa12 mutants to nucleotide-depleting agents and by the fact that both are lethal or nearly lethal in a gen5-Δ context (see below).

TFIIE is an essential component of Pol II initiation, required during the synthesis of the first few nucleotides and then ejected from the elongating enzyme (43–45). This factor is known to directly bind Pol II (41), and electron microscope crystallography has located this factor in front of the Pol II DNA channel (46). In this study, we found that Rpb9 physically interacted with the large subunit of TFIIE (Tfa1) in a two-hybrid assay, and that this binding involves a conserved domain also present in the human TFIIEα subunit. rpb9-Δ markedly reduces the co-immunopurification of Tfa1 with Pol II. Moreover, this mutant becomes lethal when combined with a temperature-sensitive allele of Tfa1, tfa1-C127W. These data strongly suggest that the interaction between Rpb9 and Tfa1 is physiologically relevant, allowing Pol II to recruit TFIIE at the edge of the DNA channel. It is tempting to speculate that the association-dissociation of TFIIE is determined by a conforma-
Myc antibodies. Enzyme prepared from the Pol II subunits (except Rpb9 itself) are present in the purified Rpb2 and anti-HA antibodies. Previous work (9) has shown that all of H9252 with invariant cysteines denoted by H9004. These constructions were tested against Gal4 AD(768–142) was fused to the full-length Rpa12 and Rpb9 and to partially deleted mutants as indicated.

Fig. 4. Two-hybrid interaction between Rpb9 and Tfa1. A, two-hybrid interaction with Tfa1. Gal4 BD(1–142) was fused to the full-length Rpa12 and Rpb9 and to partially deleted mutants as indicated. These constructions were tested against Gal4 AD(768–881) fused to Tfa1(61–164). Rpb9 and Rpa12 inserts are schematically represented with invariant cysteines denoted by black boxes. Prey and bait plasmids (described in Table I) were transformed in Y190, and a β-galactosidase assay was carried out after 3 days at 30 °C on SC-WLU medium (28). Vect., vector. B, two-hybrid interaction with tfa1 mutants. In the schematic representation of Tfa1, the shaded area corresponds to the evolutionarily conserved domain shared by all eukaryotic Tfa1. The C-terminal half (in white) is not conserved. The black boxes denote the zinc binding cysteines. The region corresponding to the insert isolated in the two-hybrid screening is indicated by a bar. tfa1 mutants (see “Experimental Procedures”) were tested for their interaction with Rpb9 using the same experimental conditions as described in the legend for Fig. 4A, C, synthetic lethality between tfa1-C127W and rpb9-D. Twenty tetrads were analyzed in a cross YYV9 (rpb9-Δ) × YSB322 (tfa1-C127W; see Ref. 27). A tetra-type tetrad illustrates the synthetic lethality of the tfa1-C127W rpb9-Δ double mutant. D, sensitivity to mycophenolate of tfa1-C127W. Wild-type (YPS500), ppr2-Δ (CMK1), and tfa1-C127W (YSB322) strains were streaked on SC and SC + mycophenolate (50 μg/ml). Plates were incubated 6 days at 25 °C.

Fig. 5. Coimmunoprecipitation of Tfa1 with Pol II. Strains YV835 (rpb2-3HA) and YV836 (rpb3-3HA rpb9-Δ) were transformed with pVV101 encoding the Tfa1–13MYC fusion. Transformants were selected on SC-W medium. Protein crude extracts were prepared as described previously (16). RNA polymerase II was immunopurified by taking advantage of the Rpb3p-3HA-tagged subunit and separated by SDS-PAGE along with crude extract controls. Pol II specific subunits (Rpb2 and Rpb3p-3HA) were revealed by Western blotting using anti-Rpb2 and anti-HA antibodies. Previous work (9) has shown that all of the Pol II subunits (except Rpb9 itself) are present in the purified enzyme prepared from rpb9-Δ cells. Tfa1–13My was detected by anti-Myc antibodies. IP, immunoprecipitate; CE, cell extract; WT, wild type.

Rpb9 Contribution to TFIIE Recruitment on RNA Polymerase II

mutant lackingRpc11 has no cleavage activity in vitro (7). TFIIIS activates this cleavage activity when recruited on Pol II (13) in a way that largely depends on the presence of Rpb9 (9). Unlike rpb9, rpc11 mutants are lethal, which led to the speculation that, in Pol III, Rpc11 may combine functions separately carried by Rpb9 and TFIIIS in Pol II (7). We show here that the growth defect of rpb9-Δ is not aggravated in ppr2-Δ cells lacking TFIIIS. In vivo, Rpb9 and TFIIIS have no additive effects on elongation. Nevertheless, it would be interesting to examine the in vivo properties of the elongating complex in these double mutants.

Our data reveal yet another facet of Pol II elongation by showing that rpb9-Δ cells fail to grow when lacking the histone acetyltransferase activity of either the Elongator (34) or SAGA complex (35). These factors may have partially overlapping functions (37), but the Elongator is thought to be associated specifically with an elongating form of Pol II (34). Gen5, the catalytic subunit of SAGA, acetylates lysine 14 of histone H3 and lysines 8 and 16 of histone H4 (48). The physiological substrate of the Elongator catalytic subunit, Elp3, is not known (to the best of our knowledge) but may also involve histone tails. Synthetic lethality need not imply a physical interaction between Rpb9 and the Elongator or SAGA factors but clearly means that there is a functional synergy between these proteins. In fact, a simple interpretation of our data is that hypoacetylated histones are an obstacle to elongation, due to the persistence of nucleosomes on the DNA template. It is known that nucleosomal templates are a poor substrate for the elongating enzyme (21, 49). Rpb9 may thus help the elongating Pol II to overcome these obstacles. This, however, is probably not a direct consequence of the RNA cleaving activity because ppr2-Δ mutants have no effect on elp3-Δ or gcn5-Δ.

Rpb9 may be part of a general mechanism allowing Pol II to sense the state of DNA (and, in particular, its degree of nucleosome packaging) prior to elongation (21) as befits the position of this subunit at the edge of Pol II, ahead of the DNA template (2). The related Pol I (Rpa12) (6, 16) and Pol III (Rpc11) subunits (7) probably have a very similar function. Our observation that Rpb9 contributes to the binding of TFIIIE onto Pol II also makes sense in this context given the role played by that factor in the transition from initiation to elongation (44, 45, 50). The molecular basis of this transition is unknown, but it probably requires a major change in Pol II conformation, associated with the opening or closing movements between the upper (Rpb2) and lower (Rpb1) sides of the DNA channel (22). By holding together the lobe of Rpb2 and the jaw of Rpb1, the N-terminal half of Rpb9 could evidently play a major role in these conformational changes. How this ultimately controls the conformation of the catalytic pocket and whether this involves the conserved zinc ribbon of Rpb9 may await further investigations.

Acknowledgments—We are especially grateful to Jean-Christophe Andraud for pointing out to us the existence of a two-hybrid response between Tfa1 and Rpb9. We thank Claire Boschiero, Olivier Gadal, Sylvie Labarre, and Benoit Van Driessche for their help during this work. Steve Buratowski for Tfa1 mutants, and Roger Sayre and Christian Marck for the RASMOL and DNA STRIDER software.

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doi: 10.1074/jbc.M107207200 originally published online January 4, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M107207200

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