A subcomplex of RNA polymerase III subunits involved in transcription termination and reinitiation

Emilie Landrieux^{1,2}, Nazif Alic¹,
Cécile Ducrot, Joël Acker, Michel Riva*
and Christophe Carles

CEA/Saclay, Laboratoire de Transcription des Gènes, Service de
Biochimie et de Génétique Moléculaire, Gif sur Yvette, France

While initiation of transcription by RNA polymerase III (Pol III) has been thoroughly investigated, molecular mechanisms driving transcription termination remain poorly understood. Here we describe how the characterization of the *in vitro* transcriptional properties of a Pol III variant (Pol IIIΔ), lacking the C11, C37, and C53 subunits, revealed crucial information about the mechanisms of Pol III termination and reinitiation. The specific requirement for the C37–C53 complex in terminator recognition was determined. This complex was demonstrated to slow down elongation by the enzyme, adding to the evidence implicating the elongation rate as a critical determinant of correct terminator recognition. In addition, the presence of the C37–C53 complex required the simultaneous addition of C11 to Pol IIIΔ for the enzyme to reinitiate after the first round of transcription, thus uncovering a role for polymerase subunits in the facilitated recycling process. Interestingly, we demonstrated that the role of C11 in recycling was independent of its role in RNA cleavage. The data presented allowed us to propose a model of Pol III termination and its links to reinitiation.

The EMBO Journal (2006) 25, 118–128. doi:10.1038/
sj.emboj.7600915; Published online 15 December 2005

Subject Categories: chromatin & transcription

Keywords: RNA polymerase III; *Saccharomyces cerevisiae*;
transcription reinitiation; transcription termination

Introduction

Under conditions of multiple round transcription by DNA-dependent RNA polymerases, initiation, elongation, and termination are the three elementary steps of the first transcription cycle, whereas reinitiation, elongation, and termination are related to the subsequent cycles. Transcription termination implies the proper recognition of the terminator elements

and the release of the newly synthesized transcript. Our knowledge of the molecular mechanisms driving termination depends on the system studied. The three transcription machineries seem to have evolved different strategies for transcription termination. Eukaryotic mRNAs possess poly(A) signals that define their 3' ends and mediate downstream transcription termination by Pol II (Proudfoot, 1989). The tight coupling of termination to mRNA 3'-end processing and polyadenylation involves the regulated interaction between 3' end processing activities and the carboxy-terminal domain of the largest subunit of Pol II (McCracken *et al*, 1997; Dichtl *et al*, 2002; Proudfoot *et al*, 2002; Zhang *et al*, 2005). In addition, chromatin remodeling of termination regions might be required for Pol II transcription termination (Alen *et al*, 2002). While the complete set of molecular mechanisms driving Pol II transcription termination remains to be fully elucidated, the requirement for the pausing of the enzyme over the terminator region to ensure termination of Pol II transcription is firmly established (Birise *et al*, 1997; Dye and Proudfoot, 2001). The mechanism of Pol I termination is different and involves *cis*- and *trans*-acting factors required to properly terminate and to release the transcript. Termination by Pol I minimally requires a sequence-specific DNA-binding protein that acts as a roadblock to pause the elongating polymerase, and a release element upstream of the pause site (Bartsch *et al*, 1988; Lang *et al*, 1994; Evers and Grummt, 1995; Zhao *et al*, 1997). In all cases, termination occurs some 10–12 bp upstream of the orientation-sensitive DNA-binding site of the termination factor. In mouse and yeast, the upstream element works in concert with a cellular factor or release factor that interacts with Pol I and drives the dissociation of the paused ternary complexes (Tschochner and Milkereit, 1997; Jansa *et al*, 1998; Jansa and Grummt, 1999). At variance with Pol I and II, Pol III accurately and efficiently recognizes termination signals constituted by a short stretch of thymidine residues in the apparent absence of any additional components (Cozzarelli *et al*, 1983). The observation that Pol III pauses at uridine stretches, and in particular at the terminator (Campbell and Setzer, 1992; Matsuzaki *et al*, 1994), strongly suggests that pausing of the enzyme is a general prerequisite for transcription by the three forms of the eukaryotic RNA polymerase. Consequently, it has been proposed that the intrinsic RNA cleavage activity of Pol III, mediated by the C11 subunit, plays a role in the termination process by raising the dwell-time of the enzyme on the terminator, and thereby favoring the release of the RNA and the dissociation of the complex (Chédin *et al*, 1998).

An important feature of yeast class III gene transcription is the direct coupling between termination and reinitiation of transcription. This process, called facilitated reinitiation, is characterized by the commitment of Pol III to reinitiate more rapidly on the same gene after the first transcription cycle

*Corresponding author. CEA/Saclay, Laboratoire de Transcription des Gènes, Service de Biochimie et de Génétique Moléculaire, F-91191 Gif sur Yvette Cedex, France. Tel.: +33 1 69 08 84 17;

Fax: +33 1 69 08 47 12; E-mail: michel.riva@cea.fr

¹These authors contributed equally to this work

²Present address: CMU/Département de Microbiologie et de Médecine Moléculaire, rue Michel Servet 1, 11211 Genève, Switzerland

Received: 7 July 2005; accepted: 22 November 2005; published
online: 15 December 2005

without being released and results in a higher initiation efficiency (Dieci and Sentenac, 1996). Facilitated recycling pathway requires the termination to take place at the natural termination signal and probably involves protein-protein interactions between Pol III and components of the preinitiation complex (Ferrari *et al*, 2004). Despite recent advances in our understanding of facilitated reinitiation, whether a particular polymerase subunit(s) is involved in this mechanism remains unknown.

In this study, we characterize an incomplete form of Pol III (Pol IIIA) lacking the C11, C37, and C53 subunits, and which displays strong transcriptional defects. Add-back experiments with combinations of the three subunits to Pol IIIA highlighted the key role of the C37–C53 complex in the recognition of the terminator elements, and demonstrated that the RNA cleavage activity of Pol III, mediated by the C11 subunit, was not involved in this step, nor in the release of the newly synthesized transcript. In contrast, the C11 subunit was required for the enzyme to perform multiple rounds of transcription. In addition, the RNA-cleavage activity of Pol III was not required for reinitiation, demonstrating a dual function for the C11 subunit. The finding that Pol III transcription termination and reinitiation require distinct, but interacting subunits, demonstrates that these two steps of the transcriptional cycle can be uncoupled. The data obtained are discussed in a model of Pol III transcription termination and reinitiation.

Results

Among the 17 subunits of the yeast RNA polymerase III (Pol III), *RPC37*, the gene encoding the C37 subunit was the last to be characterized. Peptidic sequence of internal tryptic fragments of the 37 kDa component of the yeast Pol III allowed us to identify the YKR025W open reading frame (ORF). The same ORF (renamed *RPC37*) was independently isolated in a two-hybrid screen with the Pol III subunit C53 as a bait (Flores *et al*, 1999). The interaction between the 37 kDa polypeptide and C53 was further demonstrated by pull-down and co-immunoprecipitation experiments (data not shown). The status of this polypeptide (named hereafter C37) as a genuine Pol III subunit was finally confirmed by the presence of its orthologue HsRPC5 in the human Pol III (Hu *et al*, 2002). The interaction between HsRPC5 and HsRPC53, the human orthologue of C53, is also conserved, paralleling the association of yeast C37 and C53 subunits, and is mediated by the domains conserved within their yeast counterparts (Hu *et al*, 2002). C37 was shown to be essential for yeast cell viability, similarly to all components of the class III transcription machinery. The C37 subunit has no paralogue in the two other nuclear RNA polymerases, and is therefore one of the five subunits specific to Pol III.

Mutational analysis of *RPC37*

A detailed analysis of the C37 amino-acid sequence did not reveal any conserved protein motif that might point out a particular function for the protein within the transcriptional process. Therefore, to functionally characterize this polypeptide we generated mutants by random mutagenesis of *RPC37*. Among four *rpc37* alleles isolated that conferred a thermosensitive growth phenotype, we focused our interest on the most thermosensitive *rpc37-1* allele. This allele was charac-

terized by a semiconservative mutation resulting in the I141V change, and a second point mutation introducing a premature stop codon that led to the expression of a polypeptide lacking the 27 C-terminal residues. Cells expressing this mutant version of C37 were unable to grow at 34°C (Figure 1A). To facilitate the biochemical analysis of Pol III containing the mutated subunit and to overcome the lack of specific antibodies raised against the C37 subunit (Huet *et al*, 1985), we constructed a mutant strain (C37HAΔCt) which contained the *rpc37-1* allele corrected for the semiconservative point mutation at position 141 and fused to a sequence encoding three HA epitopes at its 3' end. A control strain (C37HA) expressing the corresponding C-terminally HA tagged form of the wild-type C37 subunit was also constructed. As shown in Figure 1A, the C37HAΔCt strain was not able to grow at 34°C, similarly to the *rpc37-1* containing strain, whereas the control C37HA did not display any growth defects, indicating that the presence of the C-terminal tag did not affect growth. Collectively, these data show that the C-terminal truncation

Figure 1 Pol IIIA lacks the C11, C37, and C53 subunits. (A) Growth of the control wild-type strain (C37HA), and of the C37HAΔCt and the *rpc37-1* mutant strains at 30 and 34°C. C37 subunit expressed in each strain is illustrated schematically on the right. Dark gray—HA tag, *—point mutation. (B) Pol III purified from the control wild-type strain expressing an HA-tagged version of C37 (lane 1), from the C37HAΔCt mutant strain (lane 2), and from the SpC11-C37HA mutant strain (lane 3) were analyzed by electrophoresis on a 13% SDS-polyacrylamide gel and silver-staining. Subunits are indicated on the left, *—major contaminants. (C) Western blot analysis of Pol III (lanes 1, 2, and 3 as in B) with a mixture of antibodies directed against the HA epitope and the Pol III subunits C34 or C53. Anti-C34 antibodies are used to control even Pol III loading. (D) Rescue of the thermosensitivity of the mutant *rpc37-1* strain with plasmids overexpressing C11, C37, or C53. Corresponding empty vectors are shown as controls. Spots are formed from 10 μl of cells incubated for 2 days.

of the C37 subunit is alone responsible for the thermosensitive phenotype observed.

Identification of an incomplete form of Pol III lacking the C11, C37, and C53 subunits

To characterize the *in vitro* transcriptional properties of Pol III containing the truncated version of the C37 subunit, the enzyme was purified from the C37HAΔCt strain and from a control strain according to the usual micropurification procedure (Huet *et al*, 1996). Subunit composition of the wild-type and mutant enzymes was analyzed by SDS-PAGE followed by silver staining. The C11 subunit was not detected in the mutant Pol III (Figure 1B, lane 2). Since no antibodies against this polypeptide are available, the absence of C11 was confirmed by analyzing the RNA cleavage activity of the enzyme, which strictly depends on the presence of the C11 subunit (Chédin *et al*, 1998). Mutant Pol III purified from the C37HAΔCt strain did not exhibit any RNA cleavage activity, confirming that the C11 subunit was indeed missing (data not shown). In addition, and despite the presence of a number of contaminants at the C37 and C53 subunit level, these two subunits seemed to be absent, or at least under-represented (Figure 1B, lanes 1 and 2). To confirm these observations, Western blot analysis was performed with anti-HA antibodies (detecting the HA-tagged C37 subunit) and anti-C53 antibodies. Whereas C37 and C53 subunits were present in the control enzyme preparation (Figure 1C, lane 1), they could not be detected in the mutant Pol III (Figure 1C, lane 2). These results demonstrated that Pol III purified from the mutant C37HAΔCt strain was deprived of the three subunits C11, C37, and C53, and suggested that C37 played a role in the association of C11 and C53 within the enzyme. The effect of the *RPC37* mutation on the stability of the C53 subunit within the Pol III complex is likely explained by the direct interaction between the C37 and C53 polypeptides (see above and Flores *et al*, 1999). The destabilization of the association of C11 subunit was more surprising because no interaction could be demonstrated by two-hybrid and Far-Western between C11 and C37 (data not shown), nor between C11 and C53 (Chédin *et al*, 1998; Flores *et al*, 1999).

To confirm the existence of a link between the C11, C37, and C53 subunits by a genetic approach, we tested whether we could suppress the thermosensitive phenotype conferred by different *rpc37* alleles upon overexpression of C11 and/or C53. As shown in Figure 1D, the overexpression of the C11 subunit allowed growth of the *rpc37-1* strain at 34°C. A similar result was obtained by the overexpression of the C53 subunit (Figure 1D). This suppression was allele specific (data not shown). These genetic interactions confirmed the link between C37, C11, and C53 subunits and suggested that the thermosensitive phenotypes of the mutant *rpc37* strains resulted from a defect in the assembly and/or stability of the complete form of Pol III *in vivo*.

Interestingly, these results were reminiscent of the characterization of an incomplete form of Pol III (named Pol IIIΔ) lacking the C11 subunit and containing a substoichiometric amount of the C53 subunit (from 0 to 20% depending on the preparation), obtained upon purification of the enzyme from a yeast strain in which a deletion of *RPC11* was complemented by its *Schizosaccharomyces pombe* orthologue (Chédin *et al*, 1998). Whether the C37 subunit was present or not in Pol IIIΔ could not be determined at that time due to the

absence of specific antibodies directed against C37 and to the comigration of the C37 and AC40 subunits during electrophoresis. To address this issue, we generated a yeast strain (SpC11-C37HA) carrying an HA-tagged *RPC37* gene and a deletion of *RPC11* heterocomplemented by the *S. pombe* C11 orthologue (see Materials and methods). Western blot analysis with anti-HA antibodies and silver staining clearly showed that Pol III purified from this strain lacked the C37 subunit in addition to C11 and C53 (Figure 1B, lane 3, and Figure 1C, lane 3). Thus, the same incomplete form of Pol III is obtained from either a strain mutated in *RPC11* or in *RPC37*. From these results, we propose a new definition of Pol IIIΔ as an incomplete form of Pol III deprived of the three subunits C11, C37, and C53.

Transcriptional properties of the Pol III lacking subunits C11, C37, and C53

Pol IIIΔ was active when assayed in an *in vitro* promoter-independent transcription assay with poly [d(A-T)] as template, indicating that the C11, C37, and C53 subunits were not required for the basal process of RNA synthesis (data not shown). As previously reported, Pol IIIΔ was found to have a number of enzymatic defects *in vitro*. It initiated correctly in specific transcription assays but was deficient in termination and totally lacked the RNA cleavage activity intrinsic to Pol III. We formerly proposed the existence of a direct link between the termination defect and the absence of intrinsic RNA cleavage activity of Pol IIIΔ, but in apparent disagreement with this hypothesis, incubation of the recombinant C11 subunit (rC11) with Pol IIIΔ had no influence on its termination defect, whereas the RNA cleavage activity of purified ternary complexes was fully restored (Chédin *et al*, 1998). To understand the molecular mechanism that controls transcription termination of class III genes, we performed add-back experiments with purified recombinant C11, C37, and C53 subunits using the *SUP4* gene as a template in an *in vitro* specific-transcription assay. Note that all the results described below were identical whether the recombinant C37 and/or C53 subunits were purified from baculovirus-infected insect cells or from *Escherichia coli* cells (recombinant C11 was solely purified from bacterial cells).

The terminator of the *SUP4* gene is a T₇GT₆ sequence in the nontranscribed strand of the DNA (Figure 2A), and wild-type Pol III mostly terminated within the T₇ stretch (Figure 2B, lane 1). As previously observed, a significant proportion of Pol IIIΔ molecules read-through the T₇ stretch (hereafter called T1), and terminated within the T₆ stretch (T2). The individual addition of any of the three subunits had no influence on this termination defect (Figure 2B). In sharp contrast, when increasing amounts of purified recombinant (C37–C53) complex were added back, the enzyme terminated within the T1 stretch only, as did the wild-type Pol III (Figure 2C). The same result was obtained when Pol IIIΔ was preincubated with a mixture of the two individual rC37 and rC53 subunits (data not shown). Note that while the r(C37–C53) complex triggered a correct recognition of the *SUP4* gene terminator by Pol IIIΔ, the level of transcription was much lower than that observed for the wild-type enzyme (Figure 2C, compare lanes 1 and 5).

Overall, these data indicate that both C37 and C53 subunits play a critical role in the recognition of the terminator of class III genes by Pol III. Furthermore, they demonstrate that RNA

Figure 2 The terminator-recognition defect of Pol IIIΔ is corrected by addition of the r(C37–C53) heterodimer but not by the individual C11, C37, or C53 subunits. (A) Schematic representation of the *SUP4* terminator with the T1 and T2 blocks. (B) The products of *in vitro* transcription reactions carried out using the *SUP4* template and either the Pol IIIwt (lane 1), the Pol IIIΔ (lane 2), or the Pol IIIΔ preincubated for 10 min with an excess of recombinant C37, C53, or C11 subunits (lanes 3, 4, and 5, respectively) were separated by electrophoresis and autoradiographed. (C) Reactions as in (B) were performed with Pol IIIwt (lane 1), Pol IIIΔ (lane 2), or Pol IIIΔ preincubated with increasing amounts of the r(C37–C53) complex (lanes 3–5). Position of full-length transcripts terminated at T1 or T2 is indicated.

cleavage is not required for correct recognition of the terminator elements by Pol III.

Pol IIIΔ elongates more efficiently than wild-type enzyme

The above observations prompted us to determine the mechanisms underlying the correction of Pol IIIΔ termination defect by the r(C37–C53) complex. We have already observed that the defect in terminator recognition by Pol IIIΔ could be corrected by decreasing the UTP concentration in the transcription reaction (Chédin *et al*, 1998). The rationale for this experiment was to decrease the elongation rate of the enzyme, in particular at the terminator. Since the r(C37–C53) complex restored accurate termination to Pol IIIΔ, we examined whether this complex had an effect on the elongation rate of the enzyme. Labeled ternary complexes containing wild type or Pol IIIΔ halted on the *SUP4* gene at position +17 were generated (see Supplementary data), and purified by gel permeation to remove labeled nucleotides. Transcription elongation was next resumed upon addition of the four unlabeled nucleotides. Time course analyses of elongation of labeled 17-mer transcripts obtained with either wild-type Pol III or Pol IIIΔ in the absence or presence of the r(C37–C53) complex were performed, and labeled, full length, *SUP4*-o-tRNA^{Tyr} transcripts were visualized by gel electrophoresis and autoradiography (Figure 3A). The amounts of newly synthesized full-length transcript were quantified,

plotted as a function of time and fitted to a single exponential curve to determine the apparent rate constant (k_a) for the elongation reaction (Figure 3B). Consistent with our hypothesis, Pol IIIΔ elongated faster than the wild-type enzyme ($k_a = 0.13 \pm 0.02/s$ and $k_a = 0.047 \pm 0.01/s$, respectively). Remarkably, Pol IIIΔ incubated with the r(C37–C53) complex and the wild-type enzyme elongated with a similar apparent rate ($k_a = 0.038 \pm 0.01/s$ and $k_a = 0.047 \pm 0.01/s$, respectively). These data indicate that the r(C37–C53) complex reduces the global elongation rate of Pol IIIΔ and concomitantly corrects the terminator-recognition defect of the enzyme.

The correct recognition of the terminator is not sufficient for transcription reinitiation

The experiment described above showed that the addition of the r(C37–C53) complex to Pol IIIΔ did correct its terminator-recognition defect but resulted in a yield of *SUP4*-o-tRNA^{Tyr} much lower than that obtained with the wild-type enzyme (Figure 2C, lanes 1 and 5). This observation was puzzling, because the experiment was performed under conditions where the enzyme was limiting, and where an equivalent number of transcriptionally competent Pol IIIΔ and wild-type Pol III molecules were used (data not shown). A simple explanation would be that, in the presence of r(C37–C53), Pol IIIΔ was either simply unable to reinitiate or was affected in facilitated reinitiation despite the correction of the terminator-recognition defect. To distinguish between these two possibilities, we used an equivalent number of transcriptionally competent Pol IIIwt and Pol IIIΔ molecules (Figure 4A) to estimate the amount of *SUP4*-o-tRNA^{Tyr} synthesized by wild-type Pol III and Pol IIIΔ + r(C37–C53) during a 30-min time course, and we compared it to the amount of transcript synthesized by the wild-type enzyme under single-round conditions. Whereas the amount of transcript synthesized by the wild-type Pol III increased with time, the level of *SUP4*-o-tRNA^{Tyr} synthesized by Pol IIIΔ in the presence of r(C37–C53) reached a plateau, after approximately 5 min of transcription, which corresponded to the level of single-round transcription by the wild-type enzyme (i.e. in the presence of heparin) (Figure 4B and C). Because in the presence of the C37 and C53 subunits, Pol IIIΔ elongates at the same rate as the wild-type enzyme (see Figure 3), these results demonstrate that Pol IIIΔ + r(C37–C53) performs only a single-round of transcription on the *SUP4* gene under conditions where the wild-type enzyme carries out multiple rounds of transcription. This result suggests that the Pol IIIΔ + r(C37–C53) is affected either in transcription termination despite correct terminator recognition, or in transcription reinitiation (or in both). One difficulty is to determine, among the multiple molecular events taking place during these two consecutive steps, which of them belong to termination and which of them belong to reinitiation. For one particular transcription cycle, all molecular events taking place after the transcript release have no impact on that cycle but may affect the following cycle, and thus we consider them to belong to the reinitiation phase. Therefore, correct transcription termination only implies the proper recognition of the terminator elements and the normal release of the newly synthesized transcript. Since the above results demonstrated that Pol IIIΔ + r(C37–C53) properly recognized the terminator elements, we next investigated if it was affected in RNA

Figure 3 The r(C37-C53) heterodimer decreases the transcription elongation rate of Pol IIIΔ. (A) Time course analysis of elongation of radiolabeled 17-mer transcripts contained within the purified stalled ternary complexes of either Pol IIIwt or Pol IIIΔ to the full-length SUP4-o-tRNA^{Tyr}. As indicated, Pol IIIΔ ternary complexes were either incubated or not with the r(C37-C53) complex for 10 min. Full-length SUP4-o-tRNA^{Tyr} transcripts terminated at T1 or T2 are indicated, as well as those accumulated at the transitory pause sites (P1-P4). (B) Full-length transcripts were quantified and the values plotted as a function of time.

release. Using immobilized *SUP4* gene, we observed that the full-length transcript synthesized by the Pol IIIΔ in the presence of the C37 and C53 subunits was released in the supernatant with a timing undistinguishable from that observed when the wild-type enzyme transcribed this gene under single-round conditions (i.e. in the presence of heparin) (Figure 4D).

We conclude that Pol IIIΔ + r(C37-C53) is not affected in transcription termination, and that the reason why this enzyme performs only single-round transcription is likely to be due to a transcription reinitiation defect.

In the presence of C37 and C53, the C11 subunit, but not the RNA cleavage activity, is required for reinitiation by Pol III

The above data demonstrated that the C11 subunit was not involved in terminator recognition, nor in the release of the newly synthesized transcript. However, the observation that an enzyme lacking only the C11 subunit (i.e. Pol IIIΔ + r(C37-C53)) was not able to reinitiate transcription brought to light the importance of C11 in the reinitiation process. When rC11

alone was added back to Pol IIIΔ, we did not observe a correction of the terminator recognition nor any effect on the reinitiation level (Figure 2B, lane 5). In sharp contrast, when rC11 was added to Pol IIIΔ + r(C37-C53), which alone completely failed to reinitiate transcription (see above), the amount of newly synthesized SUP4-o-tRNA^{Tyr} transcript increased with time similarly to the wild-type enzyme (Figure 5A and B), demonstrating that Pol IIIΔ + rC11 + r(C37-C53) could reinitiate transcription. To directly address whether the capacity to reinitiate conferred by rC11 onto Pol IIIΔ + r(C37-C53) resulted in facilitated reinitiation rather than simple reinitiation, a template competition assay was performed (Figure 6A). When a limiting amount of the enzyme was added to a mixture of *GLU3* and *SUP4* tRNA genes (each preassembled into a preinitiation complex) and multiple rounds of transcription allowed, Pol IIIΔ + rC11 + r(C37-C53) equally transcribed the two templates (Figure 6B, lane 5), indicating that transcription of neither gene was intrinsically favored. In Figure 6B (lane 6), the enzyme was first sequestered in ternary complexes on the *SUP4* template, and then multiple rounds of transcription were allowed to occur

Figure 4 Pol IIIΔ + r(C37-C53) properly terminates transcription but does not reinitiate. **(A)** The number of transcriptionally active ternary complexes formed by the quantities of Pol IIIwt (lane 1) and of Pol IIIΔ (lane 2) used in subsequent experiments was assessed by the formation of stalled complexes assembled on the *SUP4* gene in the absence of GTP. Note that Pol IIIΔ incorporates an extra nucleotide at the 3' end of the nascent RNA forming an 18-mer RNA, as previously reported (Chédin *et al*, 1998). **(B)** Time course analysis of *SUP4* gene transcription performed with Pol IIIwt or with Pol IIIΔ + r(C37-C53). Single-round transcription was performed with Pol IIIwt in the presence of heparin for 5 or 30 min. **(C)** The amounts of full-length *SUP4*-o-tRNA^{Tyr} transcripts in the time course analysis shown in (B) were quantified and the values plotted as a function of time. SR—single round. **(D)** Analysis of transcript release. Full-length transcripts contained in the supernatants of transcription reactions performed on an immobilized *SUP4* template were separated by denaturing polyacrylamide gel electrophoresis, autoradiographed, and quantified. Transcription was carried out with either Pol IIIwt in the presence of heparin (single-round conditions) or Pol IIIΔ preincubated with r(C37-C53), and the reactions sampled at the times indicated. The values are given as a % of the final level of transcript released (which was similar in the two reactions examined) and plotted against time.

for 5 min in the presence of an equimolar amount of the *GLU3*-TFIIIC-TFIIIB preinitiation complex. In this case, Pol IIIΔ + rC11 + r(C37-C53) did not redistribute itself equally on both genes, as would be expected if it had been released after the first cycle. Instead, the enzyme preferentially recycled on the *SUP4* template, with the transcription of the competitor *GLU3* template being significantly excluded, as observed for the wild-type enzyme (Figure 6B, lane 4) (Dieci and Sentenac, 1996). On the basis of these data, we conclude that recycling Pol IIIΔ + rC11 + r(C37-C53) is predominantly committed to the initially transcribed gene, demonstrating that Pol IIIΔ reconstituted with the three recombinant subunits facilitated reinitiation.

We next investigated whether the requirement of C11 for transcription reinitiation of Pol III was dependent of the C11-mediated RNA cleavage activity of the enzyme. The C-terminal zinc-binding domain of C11 displays strong homology to the zinc-ribbon of the Pol II cleavage-stimulatory factor TFIIS (Chédin *et al*, 1998). To directly test the requirement for cleavage activity in facilitated reinitiation, we constructed a mutant version of the C11 protein (C11^{E92H}) equivalent to a TFIIS mutant (E291H) which was unaffected in its association with Pol II, but which did not retain any cleavage stimulatory activity (Awrey *et al*, 1998). As expected, the rC11^{E92H} protein did not confer the RNA cleavage activity to Pol IIIΔ + r(C37-C53) (Figure 5C, compare lanes 2 and 5), but did allow Pol

IIIΔ + r(C37-C53) to produce as high a transcript yield as the wild-type rC11 polypeptide (Figure 5D). Therefore, the role of C11 subunit in reinitiation is independent of its cleavage activity. In addition, this result indicates that C11 subunit plays at least two roles in the transcriptional process: a cleavage stimulatory activity and a key function in Pol III transcription reinitiation.

Discussion

This work addresses the function of the three essential subunits C11, C37, and C53 of the yeast Pol III in the transcriptional process. Reconstitution experiments of Pol IIIΔ with recombinant subunits show that the C37-C53 heterodimer is crucial for the correct recognition of the termination signals of class III genes, whereas the C11 subunit plays a central role in transcription reinitiation, independently of its well-established function in the stimulation of Pol III RNA cleavage activity.

Role of C37-C53 subunits in transcription termination of class III genes

Pol IIIΔ is defective in transcription termination, and reads through the terminator elements of class III genes. Remarkably, these defects were corrected by the addition of the r(C37-C53) heterodimer to Pol IIIΔ. The finding that the

Figure 5 The C11 subunit is required for Pol III transcription reinitiation independently of its function in RNA cleavage activity. **(A)** Time course analysis of *SUP4* gene transcription performed with Pol IIIwt, Pol IIIΔ, or Pol IIIΔ + rC11 + r(C37-C53). Single-round transcription was performed with Pol IIIwt in the presence of heparin. **(B)** The amounts of full-length *SUP4*-o-tRNA^{Tyr} transcripts of the time course analysis shown in **(A)** were quantified and the values plotted as a function of time. **(C)** Ternary complexes of Pol IIIΔ containing an 18-mer transcript were purified and incubated with wild-type rC11 or with mutant rC11^{E92H}, as indicated. RNA cleavage reaction was performed for 5 and 60 s and the products separated by polyacrylamide gel electrophoresis in denaturing conditions and autoradiographed. *—cleavage products. **(D)** *SUP4* gene was transcribed by Pol IIIΔ preincubated with r(C37-C53) alone, or with r(C37-C53) and either wild-type rC11 or the cleavage-incompetent rC11^{E92H}. Transcripts were separated by denaturing polyacrylamide gel electrophoresis and autoradiographed.

global elongation rate of Pol IIIΔ molecules was reduced in the presence of the heterodimer correlates well with the concomitant increase in the dwell time of the enzyme at the transitory pause sites of the *SUP4* gene (bands P1-P4 in Figure 3A). Efficiency of bacterial transcription termination parallels the duration of pausing, thus providing convincing evidence that the natural pause at the terminator is important for termination (Gusarov and Nudler, 1999; Bar-Nahum *et al*, 2005). This conclusion has been extended to the three forms of eukaryotic RNA polymerase (Birse *et al*, 1997; Chédin *et al*, 1998; Prescott *et al*, 2004), suggesting that the correction of the Pol IIIΔ terminator-recognition defect by the C37-C53 heterodimer arises through an increase in the dwell time of ternary complexes at the terminator, possibly reflecting a conformational change of the enzyme.

Surprisingly, correction of the terminator-recognition defect of Pol IIIΔ by the C37-C53 heterodimer did not lead to the expected increase in the amount of transcripts terminated within the T1 stretch concomitantly with the observed disappearance of tRNAs terminated within the T2 stretch. This puzzling observation was explained by the inability of Pol IIIΔ + r(C37-C53) to reinitiate transcription while Pol IIIΔ was able to reinitiate transcription, albeit less efficiently than wild-type enzyme. One explanation would be that

ternary complexes containing Pol IIIΔ + r(C37-C53) do not dissociate at the terminator. The observation that full-length *SUP4*-o-tRNA^{Tyr} synthesized by Pol IIIΔ + r(C37-C53) was released from ternary complexes with a timing undistinguishable from that observed with the wild-type enzyme under single-round transcription conditions does not support this hypothesis, but suggests that the dissociation of the ternary complexes is not affected by the absence of the C11 subunit. However, since enzyme release was not investigated, we cannot formally exclude that in the presence of r(C37-C53) Pol IIIΔ cannot dissociate from the terminator. Altogether, these results demonstrate that a correct transcription termination (i.e. recognition of the terminator elements and transcript release) is not sufficient to allow Pol III transcription reinitiation.

Role of the C11 subunit in Pol III transcription

Our data demonstrate that the C11 polypeptide is dispensable for the recognition of the terminator elements and for the release of the full-length transcript, and thus that the intrinsic RNA cleavage activity of Pol III is not involved in transcription termination, in contrast with the hypothesis raised in our previous paper (Chédin *et al*, 1998). While the dwell time of the enzyme at termination sites is indeed a key parameter for

Figure 6 Pol IIIA reconstituted with rC11 and the r(C37-C53) heterodimer performs facilitated reinitiation. **(A)** Diagram describing the template competition experiment. **(B)** The *GLU3* and *SUP4* tRNA genes were separately preincubated with TFIIC and TFIIB to allow for the formation of stable preinitiation complexes. A limiting amount (50 ng) of wild-type Pol III or Pol IIIA + rC11 + r(C37-C53) was then added for 15 min to one of the two complexes (template 1) (lanes 1, 2, 4, and 6), or to a mixture of the two preinitiation complexes (lanes 3 and 5). Incubation was continued for 5 min after addition of ATP (600 μ M), CTP (600 μ M), UTP (100 μ M), and [α^{32} P]UTP (40 μ Ci), and then GTP (600 μ M) was added alone (lanes 1, 2, 3, and 5), or together with the competitor, preassembled template 2 (lanes 4 and 6). Transcription was allowed to proceed for 5 min. The position of the two primary transcripts is indicated.

termination, the previous proposal that the multiple C11-dependent cleavage-synthesis cycles occurring at the terminator could directly determine the dwell time of Pol III at these sites is incorrect. In particular, the finding that in the presence of C37 and C53, but in the absence of C11, Pol IIIA displays the same sensitivity to transcriptional pause sites as the wild-type enzyme strongly suggests that the dwell time at the pause sites is independent of the RNA cleavage activity of the enzyme. This observation suggests that the multiple cleavage-resynthesis cycles observed at pause sites (Bobkova and Hall, 1997) are more likely to be the consequence than the cause of the transitory pausing of the ternary complexes. Rather, our current data suggest that, in agreement with data recently obtained with the bacterial RNA polymerase (Bar-Nahum *et al*, 2005), the dwell-time of Pol III at the terminator is controlled by the elongation rate of the enzyme and thus directly depends on the C37-C53 subunits. Thus, C11 has no direct effect on transcription termination, but is critical for the stable association to the enzyme of the C37-C53 heterodimer, which is required for correct transcription termination.

Remarkably, the C11 subunit fully restored the ability of the enzyme to reinitiate in the presence of the C37-C53 heterodimer. This property does not involve the C11-dependent RNA cleavage since a mutant of C11 unable to stimulate this activity still restores efficient reinitiation of the Pol IIIA in the presence of r(C37-C53). The C11 subunit has therefore two independent functions in Pol III transcription: the stimulation of the RNA cleavage activity, and a role in transcription reinitiation. Based on the chimeric structure of C11, which displays structural homologies with the Rpb9 subunit of Pol II and with the cleavage factor TFIIS (both required to efficiently stimulate the RNA cleavage activity of Pol II; Awrey

et al, 1998), we previously suggested that C11 may stimulate the Pol III RNA cleavage activity by inducing a conformational change of the enzyme allowing the positioning of the C11 Zn-ribbon at the active site of the enzyme (Chédin *et al*, 1998). We now propose that this C11-dependent conformational change of Pol III is critical for efficient reinitiation.

A model for Pol III transcription termination and reinitiation

On the basis of a quantitative analysis of RNA chain elongation on the *SUP4* gene, Geiduschek and co-workers postulated that yeast Pol III is able to switch repeatedly between a fast and a slow stepping state, possibly at each elongation step (Matsuzaki *et al*, 1994). A competition between these two states is expected to influence the global elongation rate and the termination efficiency. We propose that the C37-C53 heterodimer plays a critical role within the interconversion between these two states by favoring the slow stepping state. Thus, in the presence of these two subunits, the duration of pausing of the enzyme both at transitory pause sites during elongation and at the terminator will be increased. The first consequence is the correct recognition of the terminator elements, as observed for the wild-type enzyme or for the Pol IIIA + r(C37-C53). We propose that a second consequence would be that the C11-dependent conformational change of Pol III could occur only if the enzyme is in the slow stepping step (or if it is artificially halted on the DNA template by omission of a nucleotide). This conformational change is critical for efficient reinitiation of the enzyme either because it might be required for Pol III to release and/or because this particular Pol III conformation would be needed for a specific polymerase recapture pathway involving promoter-bound transcription factors (Ferrari *et al*, 2004). This model (Figure 7) would predict that facilitated reinitiation occurs only if the C11, C37, and C53 subunits are present. Our results indicate that the C11-dependent conformational change could be observed even with a C11 subunit having lost its ability to stimulate RNA cleavage. A mutant of C11 affected in its ability to stimulate the RNA cleavage activity but still able to induce the C11-dependent change in the enzyme conformation would also allow facilitated reinitiation in the presence of the C37-C53 subunits. In contrast, in the absence of the C11 subunit, the enzyme still correctly recognizes the terminator elements but is obviously unable to undergo the C11-dependent conformational change, staying blocked in an unproductive state for transcription reinitiation. Finally, in the absence of the C37-C53 subunits, and whatever C11 is present or not, the enzyme is in a fast stepping state, and is thus affected in terminator recognition, as observed for the Pol IIIA or for the Pol IIIA + rC11. Interestingly, in the absence of C11, C37, and C53, the Pol IIIA is able to reinitiate transcription, albeit less efficiently than the wild-type enzyme. In conditions where wild-type Pol III performs 23 cycles of transcription within 10 min, Pol IIIA performs approximately five transcription cycles. The observation that Pol IIIA elongates more rapidly than the wild-type Pol III, and that both enzymes release the full-length transcript with similar kinetics suggests that Pol IIIA is affected in facilitated transcription reinitiation. The conclusion is confirmed by the ratio of efficiency observed between the two enzymes (4.6), which is in perfect agreement with the values

Figure 7 A model of Pol III transcription termination. (A) Transcription cycle of recycling wild-type Pol III performing facilitated reinitiation. During elongation, the preferential conversion of Pol III in a slow stepping state requires the presence of the C37–C53 heterodimer. This slow state allows the correct recognition of the terminator elements, and a C11-dependent conformational change of Pol III at the termination site required for reinitiation. (B) In the absence of the C37–C53 heterodimer, the enzyme is locked in a fast elongating-conformation and poorly recognizes terminator sequences. The enzyme is able to reinitiate transcription, but not to perform facilitated recycling. (C) In the absence of C11 subunit, the enzyme is blocked in the slow stepping state. At the terminator, the newly synthesized transcript is released, but the C11-dependent conformational change of Pol III cannot occur, and the enzyme stays in a noncompetent state for transcription reinitiation.

measured when comparing facilitated reinitiation with ‘simple’ (i.e. nonfacilitated) reinitiation (Ferrari *et al*, 2004).

In conclusion, our data demonstrate that the C37–C53 subunits are required for gene terminator recognition by Pol III, and that the C11 subunit is critical for transcription reinitiation in an RNA cleavage activity-independent manner if the C37–C53 subunits are present. In addition, facilitated reinitiation of Pol III transcription requires the presence of these three subunits. Our data show that facilitated reinitiation requires the integrity of Pol III, suggesting that in addition to a conformational change of the TFIIB-DNA complex proposed by Ferrari *et al* (2004), the conformation of the Pol III itself is an important parameter for these mechanisms.

The C11, C37, and C53 subunits are intimately interconnected, as illustrated by several biochemical and genetic data.

In particular, the finding that mutations in C11 or in C37 destabilize the association of these three subunits to the enzyme (and only of these polypeptides) correlates well with immuno-electron microscopy observations showing that these three subunits colocalize within the 3D enzyme structure (C Fernandez-Tornero and C Müller, personal communication). Altogether, these results suggest that these three polypeptides belong to an autonomous structural module that encompasses polypeptides functionally important for transcription termination and reinitiation.

Materials and methods

Plasmids and mutagenesis

RPC37 was amplified from *Saccharomyces cerevisiae* genomic DNA by PCR using 30-mer oligonucleotides hybridizing downstream

(−299) or upstream (+949) of the *RPC37* ORF and containing an *EcoRI* or a *HindIII* restriction site at the 5′ and 3′ ends, respectively. The fragment was cloned into the corresponding sites of pRS316 plasmid (Sikorski and Hieter, 1989) to give pRS316-C37. pFL44-C53 (2 μm, *TRP1*, *RPC53*) was similarly constructed by cloning a PCR product containing the promoter, ORF and terminator of *RPC53* into the *KpnI* and *BamHI* sites of pFL44. pRS424-C11 (2 μm, *TRP1*, *RPC11*) was previously described (Chédin *et al*, 1998). To obtain the His₆-HA-C37 subunit, the *RPC37* ORF was amplified by PCR using the pRS316-C37 plasmid as template and primers carrying *NotI* and *EcoRI* restriction sites, and cloned between respective sites in pYeHFn2T (Cullin and Minvielle-Sebastia, 1994) to give pYeHFn2T-RPC37.

rpc37 mutant alleles were constructed by mutagenic PCR using *Taq* DNA polymerase (Amersham) in presence of 5 mM MgCl₂, and gap repair as described (Muhlrad *et al*, 1992). The plasmid for the expression of C11^{E92H} in *E. coli* was constructed by PCR-driven site-directed mutagenesis of the pGX4TK-RPC11 plasmid (Chédin *et al*, 1998). Presence of the mutation was confirmed by sequencing.

All strains used were isogenic to the yPH500 wild-type strain (Sikorski and Hieter, 1989). The *RPC37* ORF, except for the last 45 nucleotides, was disrupted with *kanMX6* cassette as described (Longtine *et al*, 1998) in the *S. cerevisiae* diploid strain yPH501 (Sikorski and Hieter, 1989). Diploid cells (*RPC37/rpc37Δ*) were transformed with the pRS316-C37 plasmid and sporulated to give a haploid *rpc37Δ* strain (yMR2) carrying pRS316-C37. pRS316-C37 was exchanged against pYeHFn2T-RPC37 in yMR2 to give yMR4. The SpC11-C37HA strain (yPH500, *rpc11::HIS3 rpc37::kanMX6*, pGEN-SpC11 (*S. pombe* C11), pYeHFn2T-RPC37) was obtained by crossing the hetero-complemented *rpc11Δ* strain (Chédin *et al*, 1998) with yMR4, followed by sporulation and spore dissection. The C37HAΔCt strain was constructed by replacing the last 81 nucleotides of the *RPC37* ORF with a sequence encoding three HA epitopes in yPH500 (Longtine *et al*, 1998). The C37HA strain was constructed by fusing three HA epitopes to C37 using the same method.

Purification of RNA Pol III and class III transcription factors

Purification of Pol III and of class III transcription factors were as described by Huet *et al* (1996). Subunit composition of Pol III was analyzed by gel electrophoresis on a 13% polyacrylamide gel under denaturing conditions (Laemmli, 1970) and proteins were silver-stained (Blum *et al*, 1987).

Expression and purification of recombinant Pol III subunits

rC11 was obtained as previously described (Chédin *et al*, 1998). His₇-C37 and C53 proteins were expressed in baculovirus-infected

insect cells and purified by liquid chromatography (see Supplementary data).

Specific *in vitro* transcription assays, elongation and cleavage assays

Plasmids used for specific *in vitro* transcription assays, reaction mixtures, and assay conditions were essentially as described (Dieci and Sentenac, 1996). Pol IIIA used was purified from the C37HAΔCt strain. Final UTP concentration was either 30 μM (experiments described in Figure 2) or 100 μM (Figures 4–6), except for the formation of 17-mer where the conditions were as described (Chédin *et al*, 1998). To examine transcript release, transcription was performed on a 557-bp biotinylated PCR product coding for the *SUP4* gene bound to streptavidine-coupled magnetic beads (Streptavidine Beads M280, Dynal) and a suitable volume of the supernatant analyzed. Purification of Pol IIIwt ternary complexes was carried out as previously described (Steinberg and Burgess, 1992). Procedure used for Pol IIIA is given in Supplementary data. Elongation assays were performed on purified ternary complexes in transcription buffer by addition of ATP, CTP, GTP, and UTP to 30 μM. Transcription under single-round condition was performed as described (Dieci and Sentenac, 1996) in the presence of heparin (100 μg/ml). For add-back experiments, recombinant proteins were incubated with Pol IIIA at a molar ratio giving the highest transcriptional signal. Transcripts were analyzed by electrophoresis on denaturing polyacrylamide gels (6% for full-length transcript and 15% for 17-mer, cleavage and elongation assays). Gels were autoradiographed using MR or MS film with or without intensifying screens (Kodak). For elongation and transcript-release assays, the amounts of total transcripts were determined, using Image Quant 1.2 software. For other experiments, quantitation was carried out using either a PhosphorImager with Image Quant (Molecular Dynamics) or an InstantImager (Packard).

Supplementary data

Supplementary data are available at *The EMBO Journal* Online.

Acknowledgements

We acknowledge Stéphane Chédin for the gift of rC11 subunit and his constant interest, and Emmanuel Favry for providing transcription factors. We thank André Sentenac for constant support and helpful discussions, and Valérie Goguel for a critical reading of the manuscript. Emilie Landrieux was supported by a short-term grant from the Fondation pour la Recherche Médicale.

References

- Alen C, Kent NA, Jones HS, O'Sullivan J, Aranda A, Proudfoot NJ (2002) A role for chromatin remodeling in transcriptional termination by RNA polymerase II. *Mol Cell* **10**: 1441–1452
- Awrey DE, Shimasaki N, Koth C, Weillbaeher R, Olmsted V, Kazanis S, Shan X, Arellano J, Arrowsmith CH, Kane CM, Edwards AM (1998) Yeast transcript elongation factor (TFIIS), structure and function. II: RNA polymerase binding, transcript cleavage, and read-through. *J Biol Chem* **273**: 22595–22605
- Bar-Nahum G, Epshtein V, Ruckenstein AE, Rafikov R, Mustaev A, Nudler E (2005) A ratchet mechanism of transcription elongation and its control. *Cell* **120**: 183–193
- Bartsch I, Schoneberg C, Grummt I (1988) Purification and characterization of TTF1, a factor that mediates termination of mouse ribosomal DNA transcription. *Mol Cell Biol* **8**: 3891–3897
- Birse CE, Lee BA, Hansen K, Proudfoot NJ (1997) Transcriptional termination signals for RNA polymerase II in fission yeast. *EMBO J* **16**: 3633–3644
- Blum H, Beier H, Gross HJ (1987) Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. *Electrophoresis* **8**: 93–99
- Bobkova E, Hall B (1997) Substrate specificity of the RNase activity of yeast RNA polymerase III. *J Biol Chem* **272**: 22832–22839
- Campbell Jr FE, Setzer DR (1992) Transcription termination by RNA polymerase III: uncoupling of polymerase release from termination signal recognition. *Mol Cell Biol* **12**: 2260–2272
- Chédin S, Riva M, Schultz P, Sentenac A, Carles C (1998) The RNA cleavage activity of RNA polymerase III is mediated by an essential TFIIS-like subunit and is important for transcription termination. *Genes Dev* **12**: 3857–3871
- Cozzarelli NR, Gerrard SP, Schlissel M, Brown DD, Bogenhagen D (1983) Purified RNA polymerase III accurately and efficiently terminates transcription of 5S RNA genes. *Cell* **34**: 829–835
- Cullin C, Minvielle-Sebastia L (1994) Multipurpose vectors designed for the fast generation of N- or C-terminal epitope tagged-proteins. *Yeast* **10**: 105–112
- Dichtl B, Blank D, Sadowski M, Hubner W, Weiser S, Keller W (2002) Yhh1p/Ctf1p directly links poly(A) site recognition and RNA polymerase II transcription termination. *EMBO J* **21**: 4125–4135
- Dieci G, Sentenac A (1996) Facilitated recycling pathway for RNA polymerase III. *Cell* **84**: 245–252
- Dye MJ, Proudfoot NJ (2001) Multiple transcript cleavage precedes polymerase release in termination by RNA polymerase II. *Cell* **105**: 669–681
- Evers R, Grummt I (1995) Molecular coevolution of mammalian ribosomal gene terminator sequences and the transcription termination factor TTF-I. *Proc Natl Acad Sci USA* **92**: 5827–5831
- Ferrari R, Rivetti C, Acker J, Dieci G (2004) Distinct roles of transcription factors TFIIB and TFIIC in RNA polymerase III transcription reinitiation. *Proc Natl Acad Sci USA* **101**: 13442–13447

- Flores A, Briand J-F, Gadal O, Andrau J-C, Rubbi L, Van Mullem V, Boschiero C, Goussot M, Marck C, Carles C, Thuriaux P, Sentenac A, Werner M (1999) A protein-protein interaction map of yeast RNA polymerase III. *Proc Natl Acad Sci USA* **96**: 7815-7820
- Gusarov I, Nudler E (1999) The mechanism of intrinsic transcription termination. *Mol Cell* **3**: 495-504
- Hu P, Wu S, Sun Y, Yuan CC, Kobayashi R, Myers MP, Hernandez N (2002) Characterization of human RNA polymerase III identifies orthologues for *Saccharomyces cerevisiae* RNA polymerase III subunits. *Mol Cell Biol* **22**: 8044-8055
- Huet J, Manaud N, Dieci G, Peyroche G, Conesa C, Lefebvre O, Ruet A, Riva M, Sentenac A (1996) RNA polymerase III and class III transcription factors from *Saccharomyces cerevisiae*. *Methods Enzymol* **273**: 249-267
- Huet J, Riva M, Sentenac A, Fromageot P (1985) Yeast RNA polymerase C and its subunits. Specific antibodies as structural and functional probes. *J Biol Chem* **260**: 15304-15310
- Jansa P, Grummt I (1999) Mechanism of transcription termination: PTRF interacts with the largest subunit of RNA polymerase I and dissociates paused transcription complexes from yeast and mouse. *Mol Gen Genet* **262**: 508-514
- Jansa P, Mason SW, Hoffmann-Rohrer U, Grummt I (1998) Cloning and functional characterization of PTRF, a novel protein which induces dissociation of paused ternary transcription complexes. *EMBO J* **17**: 2855-2864
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680-685
- Lang WH, Morrow BE, Ju Q, Warner JR, Reeder RH (1994) A model for transcription termination by RNA polymerase I. *Cell* **79**: 527-534
- Longtine MS, McKenzie AR, Demarini DJ, Shah NG, Wach A, Brachet A, Philippsen P, Pringle JR (1998) Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. *Yeast* **14**: 953-961
- Matsuzaki H, Kassavetis GA, Geiduschek EP (1994) Analysis of RNA chain elongation and termination by *Saccharomyces cerevisiae* RNA polymerase III. *J Mol Biol* **235**: 1173-1192
- McCracken S, Fong N, Yankulov K, Ballantyne S, Pan G, Greenblatt J, Patterson SD, Wickens M, Bentley DL (1997) The C-terminal domain of RNA polymerase II couples mRNA processing to transcription. *Nature* **385**: 357-361
- Muhlrad D, Hunter R, Parker R (1992) A rapid method for localized mutagenesis of yeast genes. *Yeast* **8**: 79-82
- Prescott EM, Osheim YN, Jones HS, Alen CM, Roan JG, Reeder RH, Beyer AL, Proudfoot NJ (2004) Transcriptional termination by RNA polymerase I requires the small subunit Rpa12p. *Proc Natl Acad Sci USA* **101**: 6068-6073
- Proudfoot NJ (1989) How RNA polymerase II terminates transcription in higher eukaryotes. *Trends Biochem Sci* **14**: 105-110
- Proudfoot NJ, Furger A, Dye MJ (2002) Integrating mRNA processing with transcription. *Cell* **108**: 501-512
- Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122**: 19-27
- Steinberg TH, Burgess RR (1992) Tagetitoxin inhibition of RNA polymerase III transcription results from enhanced pausing at discrete sites and is template-dependent. *J Biol Chem* **267**: 20204-20211
- Tschochner H, Milkereit P (1997) RNA polymerase I from *S. cerevisiae* depends on an additional factor to release terminated transcripts from the template. *FEBS Lett* **410**: 461-466
- Zhang Z, Fu J, Gilmour DS (2005) CTD-dependent dismantling of the RNA polymerase II elongation complex by the pre-mRNA 3'-end processing factor, Pcf11. *Genes Dev* **19**: 1572-1580
- Zhao A, Guo A, Liu Z, Pape L (1997) Molecular cloning and analysis of *Schizosaccharomyces pombe* Reb1p: sequence-specific recognition of two sites in the far upstream rDNA intergenic spacer. *Nucleic Acids Res* **25**: 904-910