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Transcription termination by the eukaryotic RNA polymerase III

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Abstract

RNA polymerase (pol) III transcribes a multitude of tRNA and 5S rRNA genes as well as other small RNA genes distributed through the genome. By being sequence-specific, precise and efficient, transcription termination by pol III not only defines the 3' end of the nascent RNA which directs subsequent association with the stabilizing La protein, it also prevents transcription into downstream DNA and promotes efficient recycling. Each of the RNA polymerases appears to have evolved unique mechanisms to initiate the process of termination in response to different types of termination signals. However, in eukaryotes much less is known about the final stage of termination, destabilization of the elongation complex with release of the RNA and DNA from the polymerase active center. By comparison to pols I & II, pol III exhibits the most direct coupling of the initial and final stages of termination, both of which occur at a short oligo(dT) tract on the non-template strand (dA on the template) of the DNA. While pol III termination is autonomous involving the core subunits C2 and probably C1, it also involves subunits C11, C37 and C53, which act on the pol III catalytic center and exhibit homology to the pol II elongation factor TFIIS, and TFIIFα/β respectively. Here we compile knowledge of pol III termination and associate mutations that affect this process with structural elements of the polymerase that illustrate the importance of C53/37 both at its docking site on the pol III lobe and in the active center. The models suggest that some of these features may apply to the other eukaryotic pols.

Keywords

RNA polymerase III; transcription termination; RPC11; RPC53; RPC37; RPC2; intrinsic transcript cleavage; oligo(U); RNA:DNA hybrid

INTRODUCTION

Transcription of a DNA template into a complementary RNA is a most fundamental process of cellular life. Bacterial and archaeal cells each use a single RNA polymerase (pol) for transcription that are evolutionarily related to the eukaryotic pols I, II and III (plants also have pols IV & V, variants of pol II) [1, 2]. Pol I transcribes a single gene type, the rRNA genes, while pol II transcribes the thousands to tens of thousands of protein coding and non-coding genes that vary over orders of magnitude in transcription output, controlled by a vast

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combinatorial set of promoters and enhancers. Pol III transcribes hundreds of tRNA genes which bear similar promoters, as well as 5S rRNA, U6 snRNA and several other noncoding RNA genes.

Transcription involves three steps: 1) initiation, -recruitment of the polymerase to the gene promoter and formation of initial phosphodiester bonds; 2) elongation, -processive synthesis of the RNA chain, albeit with intermittent pausing in some cases, and 3) termination, -cessation of RNA synthesis and dissociation of the three components: nascent RNA, polymerase, and DNA. While each of the eukaryotic pols distinguish these steps, their specialization appears to include differences in how the steps are executed, the relative time spent at each step, and how the steps may be linked to each other. For example, termination and reinitiation by pol III have been shown to be mechanistically linked [3–5]. Also, while the three pols use highly similar mechanisms of transcription initiation, there is much more complexity in assembling pol II initiation complexes than pols I & III [6]. Differences in elongation include the time spent in this mode, with pol III differing most since at 75–300 nt, its transcripts are relatively short [7]. Another difference is that pol II uses pausing as a control point, regulated by the positive transcription elongation factor-b (P-TEFb) [8].

Transcription termination must involve destabilization of the elongation complex followed by release of the nascent RNA and polymerase. This is a big transition because elongation complexes must be very stable in order to avoid the deleterious effects of premature termination [9]. Failure to terminate can interfere with downstream genes, produce 3' extended RNAs with potential adverse effects, and deplete the pool of polymerase that should be available for regulated initiation [10].

TERMINATORS SIGNAL THE INITIATION OF A TWO-STAGE PROCESS

Different RNA polymerases use different mechanisms to direct termination (Fig 1A–E). Each responds to a specific signal, a terminator element in the DNA or the elongating RNA that prompts the beginning of the termination process [11]. The process initiates with recognition of a termination signal and this is followed by induced cessation of RNA synthesis and release. Some terminators work at a distance; recognition of the termination signal by the polymerase or a trans-acting factor is the first stage and this leads, albeit more directly for some than others, to the second stage, destabilization of a paused complex with release of the DNA and RNA from the polymerase active center.

Bacterial RNA polymerase can use two kinds of terminators, factor-dependent and intrinsic [12]. Rho factor is a helicase that recognizes the nascent RNA at a C-rich sequence after it emerges from the polymerase and propels along in a 5'–3' direction to catch up with the elongation complex, inducing destabilization and termination (Fig. 1A) [13]. Intrinsic terminators work more directly, i.e., within a relatively short, albeit bipartite terminator element, to coordinate pausing and destabilization. This involves formation of a hairpin structure in the transcribed RNA followed by transcription of oligo(dA)-rich sequence to produce an RNA with an oligo(U)-rich 3' end [12, 14]. Termination occurs as the oligo(rU:dA) hybrid is melted in the active center of the polymerase (Fig. 1B).

Pol II termination is more complex, involving post translational modifications of the polymerase as well as association of a number of trans-acting factors [10, 15]. Similar to bacteria different termination signals distinguish at least two types of pol II termination mechanisms, for poly(A)-containing mRNAs and for poly(A)-independent small nuclear RNAs [16]. For the first type, the AAUAAA poly(A) addition site in the elongating RNA recruits a complex of factors that endonucleolytically cleave the transcript which is then further processed to become a mRNA. This is followed by 5'–3' exonucleolytic digestion of the segment of RNA still attached to the polymerase (Fig. 1C). Thus for most pol II-

transcribed mRNA genes the termination signal is the poly(A) addition site in the newly synthesized RNA which acts at a distance; its recognition by RNA-binding factors initiates a process that ends in termination at a downstream site.

Pol I uses a termination factor, Ydr026C/Nsi1 bound to a specific element on the downstream DNA [17] as well RNA endonucleolytic cleavage followed by 5' exonucleolytic digestion [18–20]. Pol I transcript release occurs within an oligo(dA) tract on the template DNA [21] (Fig. 1D). According to a current 'torpedo' models of termination by pols I & II, the 5'-3' exonucleases catch up with the elongating polymerases to induce pausing and destabilization leading to the final stage, release, reminiscent of Rho-dependent termination [11, 20].

Pol III has a most direct acting termination signal, more similar to but distinct from intrinsic termination by bacterial RNA polymerase, than pols I or II. Oligo(dA) on the template DNA is sufficient to commence and complete all steps leading to termination by pol III (Fig. 1E) [22–24]. The pol III enzyme incorporates five stably associated subunits, the heterotrimer C31/34/82 and heterodimer C37/53 as two subcomplexes with homology to the pol II ancillary factors, TFIIIE and TFIIF, as well as C11, a two-domain polypeptide with homology to Rpb9 and the elongation factor TFIIS, that collectively promote efficient initiation, termination and reinitiation (Fig. 2A, B) [25–30]. Unlike initiation by pol III which can be directed by gene types that differ in promoter structure and the transacting factors that recognize them, termination appears to occur by the same basic mechanism for all (with exceptions that likely reflect variations on the oligo(dT) theme; see below). These characteristics make pol III an attractive model for the study of the mechanism of termination by a eukaryotic RNA polymerase.

In summary, the eukaryotic RNA polymerases use different signals to initiate the termination process. It is much less clear as to how their active centers work to execute the final stage of termination, release from the RNA and DNA by their active centers, and to what extent they may share mechanisms involved in this stage of the process.

TERMINATION BY POL III: THE SIGNAL IS OLIGO(dT)

Comparison of the 3' oligo(U) sequence at the ends of 5S rRNA with the 3' regions of *Xenopus* 5S rRNA genes suggested that a stretch of 4 or more T residues in the DNA could act as a pol III terminator [31–34]. It was shown that pol III in *Xenopus* oocytes or in solution would terminate at oligo(dT) at the 3' ends of 5S rRNA genes [22, 24].

While independent studies confirmed oligo(dT) as a universal terminator for pol III, further analysis revealed species-specificity in the minimum length of the oligo(dT) tract required for termination. For vertebrates, as few as 4 Ts can act as an efficient terminator while for yeasts variably longer T tracts are required [22, 24, 35]. In general, *S. cerevisiae* requires 6 or more Ts [36–38] while *S. pombe* requires 5 or more Ts, for efficient (~90%) termination [37], and this is supported by genome-wide analyses of tRNA genes [38, 39]. It was further suggested that fission yeast *Schizosaccharomyces Japonicus* requires only 4 Ts at a large number of tRNA gene terminators [39]. It is expected that these differences are manifested in sequence disparities in the termination-relevant regions of the pol III subunits of these species. Noteworthy is that the minimal T length requirement of the different species correlates with the α -amanitin sensitivities of their pols III [37], the implications of which will be discussed in a later section.

Oligo(dT) flanking sequence context effects

Early studies revealed that the sequence surrounding the oligo(dT) signal can influence termination efficiency [22, 24]. This effect may be most pronounced for vertebrate pol III for oligo(dT) length of 4 [40]. 4Ts flanked by AA is very inefficient as a terminator, and similar to *Xenopus*, most human pol III will read through, whereas replacing AA with GC increases termination efficiency dramatically [40]. By contrast, a 5T tract is highly efficient for pol III termination [22, 24] and relatively insensitive to flanking sequence [40]. Nonetheless, a 4T terminator flanked by GC can be as efficient as a 5T terminator [40]. This context-dependency of 4T tracts is critical for some pol III transcribed genes with 4Ts in their coding region, such as all lysine tRNA genes and adenovirus VA RNA_{II} [41]. One may suspect that the necessity for four contiguous Ts in lysine tRNA genes (the invariant U33 in the mature tRNA followed by the UUU anticodon at positions 34–37) may have set an ancient limit of no fewer than 5Ts as the minimal pol III terminator which was later adjusted in multicellular eukaryotes by the ability to utilize sequence-context effects. In *S. cerevisiae* a 5T stretch is relatively inefficient and can act as a terminator only in the right context [38] although the magnitude of the effect of flanking sequence may be less than on a 4T terminator for human pol III. However, there are no hard ‘universal’ rules with regard to flanking sequence. In *S. cerevisiae*, CT following the T5 tract can weaken the terminator while A or G strengthens it [38]. Also, termination efficiency of a *Xenopus* lysine tRNA gene with a 4T terminator was influenced by flanking sequence differently than for *Xenopus* 5S rRNA genes [42]. Notably, these sequence context effects do not require other factors as pol III itself from yeast or *Xenopus*, exhibited context dependent termination [24, 38].

Flanking sequence effects might suggest the involvement of secondary structure. Although proximal hairpins appeared to promote pol III termination in one study [43], others indicate no such effects, confirming oligo(dT) alone as the major terminator element for pol III. Later in this review we note that for some genes, flanking sequence effects may extend to binding sites for extraneous factors that influence termination, e.g., the NF1 site adjacent to the terminator of the VA1 RNA gene [44].

While oligo(dT) is clearly the most prevalent terminator for pol III, isolated reports cite non-canonical pol III terminators, and although most of these are interrupted oligo(dT) tracts, one was a run of A residues downstream of a mouse 5S rRNA gene [45]. Human Pol III was reported to terminate 3′ of an Alu repeat which did not have 4 or more consecutive T residues and the potential for RNA hairpin formation was noted [46]. However mapping pol III terminators using cellular extract can be complicated. Following *in vitro* transcription of a B1-Alu gene element using cellular extract, 3′-5′ digestion of the nascent RNA was so robust that mapping of the terminator that gave rise to the barely detectable primary transcript required rapid pulse-chase conditions [47]. Remarkably, this robust 3′ digestion occurred following transcription of the B1-Alu gene that had a 4T terminator but not the same gene with a 5T terminator, and this was later attributed to the 3′-protective activity of the 3′ oligo(U) length-specific RNA binding protein, La [48]. Another case is the avian adenovirus CELO VA RNA gene; TTATT caused inefficient termination [41]. Termination within a T stretch shorter than 4 Ts or an interrupted T stretch has been noted for a human tRNA^{met} gene [49].

As noted above and discussed later in more detail, a large number of human tRNA genes that use non-canonical terminators have been catalogued but these are variations of oligo(dT), generally a T3, T2 or T1 stretches separated by another nucleotide (interrupted T5 or T4) [50]. These studies together with the effects of flanking sequence on termination efficiencies suggest a complex mechanism(s) that control the efficiency of pol III termination on short oligo(dT) terminators.

Primary and secondary oligo(dT) terminators

Bacteria use a variety of anti-termination mechanisms to control the polar inclusion or exclusion of segments of polycistronic mRNAs [12]. An intriguing concept for pol III is that of secondary terminators, i.e., an oligo(dT) stretch downstream of a weak primary terminator that can be used to produce a unique longer transcript. In one scenario, a fraction of pol III would terminate at the first oligo(dT) while the rest would read through to terminate at the downstream terminator [22, 33, 49]. Human adenoviral VA RNA_I and avian adenovirus CELO VA RNA genes provide examples that can produce two different RNAs by this mechanism [41, 51].

Recent identification of biological functions for tRNA 3' fragments derived from the 3' trailer sequences of pre-tRNAs suggests that partial read through of the first terminator may be used to generate such tRNA 3' fragments [52–54, reviewed in 55]. In accord with this, Orioli et al. identified a large number of tRNA genes in humans with weak terminators followed by a strong secondary terminator [50]. A fraction of pol III terminates at the weaker terminators while the rest read through. These observations further suggest the exciting possibility that pol III termination may be modulated to produce a subclass of non-coding RNAs, although this remains to be determined. A genome wide chromatin immunoprecipitation (ChIP) followed by sequence analysis of human pol III showed that a significant fraction of pol III was unexpectedly found to be accumulated somewhat downstream of the tRNA gene terminators which may reflect among other things terminator read-through and/or a state of pausing [50].

THE TERMINATION MECHANISM

In simple accordance with a kinetic coupling model in which termination efficiency is inversely related to elongation rate, pausing provides a window of time during which alterations can occur in the polymerase that lead to transcript release [56, 57]. Indeed, RNA polymerase pausing at a terminator is prerequisite for termination [23, 58–60]. Kinetic coupling is supported by the fact that methods that slow elongation lead to increased termination efficiency [25].

While oligo(dT)-rich tracts are recognized as pause sites, they are not sufficient to cause termination by other RNA polymerases which must traverse many kilobases (for pol II megabases) that often contain oligo(dT) by chance or necessity. The bacterial polymerase terminates when an oligo(dT)-rich tract closely follows a hairpin in the transcript. This arrangement increases specificity by enriching the signal with more complexity than oligo(dT) and allows both elements to function toward destabilization of the complex within the confines of the active center and adjacent elements [59].

Multiple hypotheses may explain the mechanism(s) of action of the hairpin during intrinsic termination. A forward translocation model suggests that hairpin formation induces polymerase to translocate forward without nucleotide incorporation, leading to shortening and destabilization of the hybrid and transcript release [61]. A second model suggests that the hairpin induces melting of the proximal end of the hybrid and destabilization [62]. A third suggests an allosteric mechanism with direct interaction of the hybrid with the polymerase trigger loop inducing structural changes that loosen the grip on the hybrid, hybrid melting and destabilization of the complex [13].

Termination must overcome the stability of the elongation complex

One of the most important features of efficient transcription is the very high degree of processivity of RNA polymerases, without which there would be an overwhelming number of incomplete transcripts [9, 63]. Poor processivity would not only be wasteful but probably

also cause havoc to RNA processing systems. Thus by design RNA polymerases must be processive and this is achieved via highly stable grips on the RNA and the DNA. Part of this stability is rooted in the complementary base pairing interactions that hold together the transcribed RNA and the DNA template within the active center of the polymerase [64, 65]. Elegant studies of bacterial RNA polymerase and pol II have shown that the nascent RNA forms an 8–9 nt hybrid with the template DNA [64, 65]. A strong RNA:DNA hybrid is one of the primary elements that is critical to the remarkable stability of the elongation complex and its processivity [65–67]. Thus the termination process must destabilize a very stable elongation complex, and it must do it precisely on cue and only on cue. As reviewed below it appears that many if not all RNA pols take advantage of a similar characteristic to do so, the outstanding weakness of rU:dA hybrid base pairs [68].

The weak rU:dA hybrid is likely an underlying component of pol III termination

Oligo(dT) (oligo(dA) in the template) constitutes a part of the termination complexes of bacterial, phage, archaeal, eukaryotic, and viral RNA polymerases [11, 12, 21, 69, 70]. The demonstration that a (rU:dA)₅ hybrid is at least 200 times less stable than the corresponding hybrid containing (rA:dT)₅ or other sequences, led to the proposal that a decrease in the stability of the hybrid as it acquires rU:dA richness may be a driving force for termination [68]. A weak hybrid such as oligo(rU:dA) causes alterations of the elongation complex such as pausing followed by backtracking [64, 71, 72]. Shortening is an alternate means of hybrid weakening and this is also implicated in transcript release by bacterial RNA polymerase [62].

As alluded to above, pol III termination appears to be somewhat similar to intrinsic termination by bacterial RNA polymerase. Intrinsic terminators are bipartite, with a 7-to-8 nt T-rich tract preceded by a G+C-rich dyad repeat that forms a stem-loop hairpin in the nascent RNA upstream of the 3' U-rich tract [59, 61, 73–76]. While an encounter of either a T-rich tract or a G+C-rich dyad may in some contexts cause pausing, both are required for complete termination [73]. Yet only an oligo(dT) tract (dA in the template) is required for pol III termination, but not a dyad-repeat, hairpin, or other cis- element. This suggests that pol III may be exquisitely sensitive to the destabilizing effects of an oligo(rU:dA) hybrid, a feature that could be accommodated by a polymerase whose substrate genes are short enough as to not require internal T tracts. The challenge is to understand the mechanisms that provide pol III the ability to respond so dramatically and efficiently to a simple signal that induces both pausing and destabilization.

Although there is no direct evidence that rU:dA hybrid weakness provides a mechanism of pol III termination, some observations support this. Replacement of rU with Bromo-rU, which forms a more stable hybrid with dA, in *in vitro* transcription reactions promotes terminator read through [63]. Lower reaction temperature which among other things increases hybrid stability, increases terminator read through [22]. Also, the template strand oligo(dA) is required for termination while the non-template oligo(dT) can be mutated with little effect (A.G.A. & R.M., in preparation).

The exact position at which pol III releases its RNA from within the oligo(dA) tract is heterogeneous as reflected by a variable number of 3' Us on the RNAs released from a single gene [57, 63, 77]. A positive correlation between oligo(U) length and transcript release can be discerned [57], consistent with the idea that longer rU:dA hybrids destabilize the elongation complex leading to better termination.

These observations support the idea that an extensive rU:dA hybrid destabilizes the elongation complex and promotes termination. However, none of them exclude the

possibility that a contributing influence may be due to sequence-specific recognition of the rU:dA hybrid with allosteric alteration of pol III as an underlying mechanism.

Is backtracking involved in pol III termination?

A common response of RNA polymerases upon encountering oligo(dT) is pausing, followed by a more stable block to elongation caused by backtracking, a process in which the polymerase 'backs-up' i.e., slides upstream while maintaining a ~10 bp RNA:DNA hybrid in the active center [64, 71]. During backtracking the RNA 3' end is displaced from the catalytic site and ejected out the secondary channel of the polymerase. RNA synthesis can be restarted from this state after the catalytic center is converted to an endonucleolytic transcript cleavage site by the cleavage factor TFIIS for pol II and the GreB and GreA factors for bacterial RNAP [78–80]. However, polymerase backtracking that is unresolved by transcript cleavage remains in a state of transcription arrest. It was proposed that backtracking may be involved in termination by pol III [81]. Certainly pol III undergoes 3' retraction, i.e. e., the cleavage of 3' terminal residues from RNA in some cases allowing otherwise stalled complexes to move forward [25, 82–84]. Whether pol III undergoes backtracking as part of termination is an outstanding question.

POL III SUBUNITS INVOLVED IN TERMINATION

The 17 subunits of pol III are intricately connected via an extensive interaction network [85]. Experimental data indicate that several pol III subunits can affect the termination process, C2, C11, C37 & C53, and possibly C1 and others [25, 26, 29, 86–89]. In addition, there have been multiple reports that extraneous factors can also affect termination by pol III (below). A challenge is to determine the mechanisms used by these subunits and extraneous factors to affect termination and to distinguish between direct and indirect (e.g., allosteric effects via other subunits) effects.

The pol III active center is organized from parts of several subunits

For the purposes of this review we shall refer to the active center as that which contains the catalytic site and its multiple structural elements such as bridge helix, trigger loop and fork loops that comprise the catalytic site responsible for nucleotide selection and phosphodiester bond formation, as well as the adjacent RNA:DNA hybrid of nearly ten base pairs (Fig. 2C). By comparison of electron micrographs of pol III with crystal structures of pol II it seems clear that the core structures of these are conserved (Fig. 2A). Indeed C1 and C2 are most homologous to their pol II homologs along the catalytic center including the invariant NADFDGD motif in the largest subunits of all multisubunit RNA polymerases, and surrounding regions [90, 91]. Thus we can be reasonably sure that RPC2, the second largest subunit of pol III together with the largest subunit C1, form an extensive active center similar to but with distinctive differences from pol II [6, 28, 91–93]. Certainly, the core pol II structure fits very well into the electron micrograph envelope of pol III (Fig. 2B) [6, 28, 91, 93].

Peripheral surfaces of pol III such as the jaws and lobe are in proximity to incoming DNA as polymerase moves along the template (Fig. 2B) [91]. While the dimerization domains of the C37 and C53 polypeptides have been localized to a bulge comprising part of the upper jaw adjacent to the lobe, biochemical and physical evidence indicate that other parts of these proteins extend into the catalytic site. A region downstream of the dimerization domain of *S. cerevisiae* C37 was localized by physical proximity methods near the active site *in vitro* [94] and the homologous region of *S. pombe* C37 is a hot spot for mutations that impair termination *in vivo* [30]. In addition, a region of C11 with strong homology to elongation factor TFIIS likely inserts into the active site to mediate intrinsic transcript cleavage with

effects on RNA 3' end formation during termination [25, 26, 29, 88]. Part of C53 lies close to the RNA 3' end in the catalytic center of the pol III elongation complex [95]. These observations suggest that the pol III active center is a busy place during termination, comprised of parts of multiple subunits. As might be expected for an extended active center and peripheral nucleic acid-interacting motifs, more than one region of the C1 and C2 subunits would also be involved in termination.

The pol III lobe is involved in termination probably via C53/37 and C11

A major advance toward understanding pol III termination came from a genome-wide screen in *S. cerevisiae* to identify genes that affect termination led to isolation of *ret1-1*, a transcription mutant with a mutated allele of RPC2 [86, 96]. *ret1-1* was selected as a mutant whose pol III could read through an otherwise functional oligo(dT) terminator placed within the intron of a suppressor tRNA gene [86].

Three regions of RPC2 were then chosen for random mutagenesis and selection for either gain or loss of function, yielding alleles with mutations that either increased or decreased termination *in vivo* and *in vitro* [87]. Further analyses revealed that many of these C2 termination-altering mutations also increased or decreased the intrinsic RNA 3' cleavage activity of pol III suggesting a natural relationship between termination and RNA 3' cleavage [83].

The three regions of RPC2 were chosen based on termination mutants in the second largest subunit of *E. coli* RNA polymerase [97]. Of particular interest is the *E. coli* polymerase β 500–575 which overlap with the RPC2 455–524 region and *E. coli* β 1230–1342 region that overlap with the RPC2 1061–1081 [87]. Termination altering mutations in similar regions of bacterial and eukaryotic RNA polymerases indicate crucial roles in termination in general [87, 97] and implies that all multisubunit RNA polymerases may undergo similar changes during transition from elongation to termination.

Although the 3-dimensional locations of the three pol III regions were unknown at the time of their analyses, presently available structures of *S. cerevisiae* pol II and pol III allow their localization to three conserved elements referred to as the lobe, fork loops and anchor regions [87, 90] (Fig. 2B). Mutations in lobe residues 300–325 of *S. cerevisiae* RPC2 are generally associated with decreased termination as is the loss of function mutant, *ret1-1*: T311K [86]. Curiously, a strong gain of function mutation in this region is K310T suggesting that threonines at either 310 or 311 promote termination.

An unbiased genetic screen of zebra fish for digestive system disruption uncovered a deletion in C2 corresponding to aa 259–300 in *S. cerevisiae* C2 that caused a developmental malformation referred to as the *sjm* (slimJim) mutant [89]. Recapitulating this deletion in *S. pombe* C2 led to dissociation of C11 from pol III confirming that this region is required for efficient C11-C2 interaction [89] (Fig. 2) [also see Fig 5 in 29]. Most remarkably, overexpression of zebra fish C11 reversed the gross digestive anomaly, attributing the phenotype caused by the C2 mutation to decreased association of C11 and C2 [89]. Since association of C11 is also required (in yeast) for stable association of the C53/37 heterodimer [88], the *sjm* mutation may further compromise the zebra fish pol III. This study, which used molecular modeling based on comparisons of yeast pols II and III strongly supports the idea that the RPC2-RPC11 interface is similar to the RPB2-RPB9 interface and functionally conserved from yeast to vertebrates [89]. In addition, *sjm* was the first to reveal that mutations in a pol III subunit, a housekeeping enzyme, can have devastating tissue-specific phenotypic effects [98], including in humans [99–102].

Therefore the *sjm* deletion, corresponding to ScRPC2 259–300, together with the ScRPC2 300–325 mutations identified by Shaaban et al. [87] form a contiguous region extending from a C11-interacting surface to what appears to be the upper ridge of the pol III lobe adjacent to the cleft from which incoming DNA moves into the active center (Fig. 2B, also see figure 5C–G in [29]). This region likely affects the association of C11 and the C53/37 termination subcomplex with pol III.

In remarkably good agreement with the structural localization of C53/37 dimerization domains on pol III are physical proximity data (photo-crosslinking and Fe-BABE mediated cleavage) [94]. The lobe region of C2 that harbors termination mutations was extensively cross-linked to the dimerization domains of C53/37 including numerous interactions between C37 and C2 303–329, as well as between C53 and C2 276–280 [94], suggesting the lobe as docking site for C53/37 adjacent to the C2 259–300 region that interacts with the Rpb9-homologous domain of C11 (see [29]).

The fork loops of C2 in the pol III active center are involved in termination

A large number of *ret1* mutations occurred in a conserved region of C2 encompassing fork loop 2, the majority of which reduced termination [87] (Fig. 2C). The fork loops are mobile elements that play critical roles in RNA strand separation and maintenance of the transcription bubble [103, 104]. Deletion of fork loop 2 from pol II is known to affect the rate of catalysis by impairing sequestration of substrate NTPs and to cause increased pausing and elongation arrest [105, 106].

The pol II fork loop 2 is a very highly conserved “IGRDGKLA” motif spanning aa 502–509 of *S. cerevisiae* RPB2. Structural studies of pol II had shown that the tip of the loop, containing RDGK, is mobile and was captured in two conformations, interacting with either the bridge helix or the non-template DNA [107, 108]. The positively charged side chains at the positions occupied by Arg (R) and Lys (K) are conserved by all multisubunit RNA polymerases and are suggested to be involved together with an invariant Arg 512, in DNA strand separation as well as NTP binding and sequestration [106]. The corresponding region, “FEKT/SRKVS” spanning aa 477–484 of *S. cerevisiae* C2 is highly conserved in pols III but divergent from pol II. Mutations of E478 of *S. cerevisiae* C2 which is conserved by pols III affects termination [57, 87]. Substitution with Lys increased while Asp decreased termination [57, 87].

The region between fork loops 1 and 2 has an imperfect tripeptide repeat in which every third amino acid is hydrophobic. Most mutations to these hydrophobic residues led to increased termination suggesting that a hydrophobic network in this region contributes to maintaining stability of the elongation complex [57, 87].

Another advance in pol III termination was the physical mapping of a region of C37 to the fork loops and other elements near the catalytic site [94]. Evidence that this was relevant to termination came from transcription analysis of a mutated form C37 that a 5-amino acid tract deletion (scC37 226–230) surrounding the fork loop and (β DloopII) interaction site [94]. Pol III reconstituted with the mutated C37 read through the SUP4 tRNA gene terminator significantly more than did the nonmutated enzyme [94].

The anchor region of C2

All mutations in the anchor region of C2 led to increased termination. The anchor region of pol II connects the RNA:DNA hybrid-binding domain to the clamp and lies between flexible switch domains 3 and 4 that control the mobility of the clamp [109] (Fig 2B). The role of the anchor region in pol III termination is currently unknown although it is expected to control allosteric alterations.

Results of *in vitro* transcription with the C2 mutants generally adhered to the kinetic coupling model; faster elongation rate was associated with reduced termination and slower elongation was associated with increased termination [57, 87] although some exceptions are noteworthy. Very interesting is the C2 double mutant T455I, E478K. As a single mutation, T455I shows increased elongation, decreased termination and efficient RNA release [57, 87]. Not surprisingly, the equivalent *S. pombe* mutant in *rpc2-T455I* also exhibits decreased termination [29, 30]. The single E478K mutant has slow elongation with increased terminator recognition (i.e., pausing in the terminator) [87] but poor release of RNA [57]. When these mutations were combined as in T455I, E478K, the net effect was decreased elongation and decreased termination, apparently uncoupling the kinetics of elongation rate and termination. In this case uncoupling occurred in association with decreased RNA release, reminiscent of the effects of nonreleased RNA on the uncoupling of pausing and termination [23]. However, these two cases are different; for E478 mutants, the pause was more like an arrest. In any case, according to the model, E478 would be in fork loop 2 while T455 would flank fork loop 1, in close proximity to the RNA backbone of the RNA:DNA hybrid. Another mutant that uncouples elongation rate and termination is C2 K512N [87]. The corresponding pol II residue shows extensive interactions with others in the region suggesting an intricate interaction network [109]. So, a mutation here might affect the integrity of the region leading to the termination defect.

In summary, it is remarkable that nearly 15 years after mapping these *S. cerevisiae* C2 mutants, the same regions were identified as in close proximity to the C53/37 termination subcomplex [94]. The lobe region shows extensive crosslinks with the dimerization domains of C53/37 suggesting the lobe as a docking site of C53/37 adjacent to C11. The C terminal region of C37 was cross-linked to the fork loop region where another set of mutations were observed. Careful analysis of the C2 mutants revealed that most mutations in the lobe produced reduced termination which now are most likely due to decreased association of C53/37. Mutations in the region 455-524 that encompass fork loop 2 led to either increased or decreased termination reflecting a pivotal role in setting a balance between elongation and termination. Mutations in the anchor region produced only increased termination suggesting that mutations in this region could be destabilizing the elongation complex.

C1 and potential effects of α -amanitin on pol III termination

α -amanitin is a fungal cyclic octapeptide that inhibits transcription by eukaryotic RNA polymerases to varying extents [110, 111]. It was noted that α -amanitin termination by *Xenopus* pol III although this remains unsubstantiated [22]. Co-crystallization with pol II has shown that α -amanitin is bound via interactions with the bridge helix and trigger loop, trapping the latter and limiting its mobility [112, 113]. While pols II are typically most sensitive to amanitin, pols III show species-specific sensitivities. *S. cerevisiae* pol III is quite resistant, vertebrate pol III is more sensitive and *S. pombe* pol III shows intermediate sensitivity [37]. Intriguingly, these amanitin sensitivity patterns correlate with the oligo(dT) minimal length requirement for termination: human pol III is most sensitive and requires fewer Ts, *S. cerevisiae* pol III is least sensitive and requires the longest T tract, and *S. pombe* is intermediate in both amanitin sensitivity and T length [37]. This correlation suggests that the sequence differences between different pol III species in the bridge helix and trigger loop might contribute to termination. These elements are involved in forward translocation of polymerase and thus constitute motifs that determine elongation rate [113–115]. Mutations in this region of C1 affect pausing, RNA cleavage and transcriptional transitions [116]. Random mutagenesis followed by *in vivo* screening of this region of C1 for termination mutants might reveal further insight into mechanisms of termination by pol III.

C53/C37: A dynamic duo

Another major advance in pol III termination came from characterization of a C11 mutant that produced pol III enzyme devoid of C11 as well as C53 and C37, known as pol III Δ [25, 88]. It was shown that the C53 and C37 subunits are required for oligo(dT) terminator recognition (pausing) and form a stable heterodimer whose interaction domains are attached to the surface of pol III near the leading edge of incoming DNA close to the Rpb9-homologous domain of C11, [28, 88, 91, 92, 94]. The C53/37 dimerization domains are homologous to the dimerization domains of transcription initiation factor TFIIF β/α which appear to occupy a similar surface on pol II [27, 94]. While these domains hold C53/37 to a surface location, biochemical evidence indicate that other parts of these polypeptides reach into the pol III active center.

Association of C53/37 with pol III is dependent on C11. Purified pol III fails to terminate at some terminators and also lacks the intrinsic transcript 3' cleavage activity [88]. Add back experiments show that recombinant C11 restored cleavage activity but not termination whereas C53/37 restored terminator recognition [25, 88]. Kinetic analysis suggest that C53/37 reduces the elongation rate of pol III on the SUP4 tRNA gene such that pol III Δ appears resistant to several gene-internal pause sites [88]. This suggested a mechanism by which C53/37 promotes termination, by reducing elongation rate thereby increasing the pause or residence time of pol III on the terminator, consistent with kinetic coupling. In accordance with this model, reducing the pol III Δ elongation rate by decreasing the NTP concentration corrects the termination defect [25, 88].

Although this model is nicely supported by experimental data, there are some notable points. First, the decreased elongation rate caused by C53/37 is manifested as pausing throughout the gene not only at the terminator [88]. Thus, while reduced elongation rate is consequential to termination according to kinetic coupling, it is not specific to the terminator. Another indication that C53/37 action is not specific to termination is evidence that it can function in initiation by participating in open complex formation similar to certain transcription initiation factors including its pol II homolog, TFIIF α/β [94, 95]. Thus, C53/37 appears to be quite dynamic in the breadth of its activities. Moreover, related to both this activity and termination is the requirement for C53/37 along with C11 for facilitated reinitiation by pol III, which is mechanistically dependent on termination [3, 4, 88, 117]. Facilitated reinitiation dependent on termination has also been observed for mammalian pol III [40, 44, 48, 118–121].

Facilitated reinitiation, which has been observed *in vitro*, is a most appealing process because it can account for the extraordinary efficiency with which pol III and the TFIIB/C stable transcription complexes can be recycled to produce the large amounts of tRNAs, 5S rRNA and other components required for cellular proliferation [122, 123]. Facilitated reinitiation. A recent study examined for the first time a potential link between termination deficiency and overall transcription output. The results showed that termination deficiency was not accompanied by a decrease in transcription output and question the degree to which if any, a link between termination and recycling observed *in vitro* is operational *in vivo* [30]. More studies will be needed to address the mechanisms by which pol III is able to reinitiate with apparent high efficiency *in vivo*.

Photo cross-linking and other physical methods revealed key interactions between C37 and C2 as well as other subunits [94]. As noted above the dimerization domains of C53 and C37 reside near the lobe domain of C2 and the Rpb9-homologous region of C11 (Fig. x) [28, 92, 94]. Residues 226–230 in the C terminal region of C37 far downstream of its dimerization domain were found to react in the immediate vicinity of C2 fork loops 1 and 2, β DloopII

and a hybrid binding region in the active center of pol III [94]. Deletion of this tract from C37 led to deficiency of pol III termination *in vitro* [94].

In support of function of the C-terminal region of C37 in termination, are point mutations in multiple residues in a homologous region of *S. pombe* C37 mutants isolated from a genetic termination screen [30]. The mutants produced increased terminator read-through transcripts from various tRNA genes *in vivo* [30]. The observations that C37 residues localize with C2 fork loops and that C2 fork loop mutations had been independently isolated as termination mutants argue that these regions participate in termination. Moreover, proximity of these regions of C2 and C37 to the pol III active site further suggests that termination involves alterations to catalytic activity that are more complex than passive cessation of phosphodiester bond formation [30] (below).

The cumulative data suggest that C37 contributes to and modulates the active center of pol III [30]. As such, C37 may be considered as one of several polypeptides that can access the catalytic site of an RNA polymerase to affect its activity, the prototypical examples of which are TFIIS for pol II and its bacterial counterpart GreB [80, 124]. However, while TFIIS and presumably the homologous cleavage-domain of C11 access the catalytic center through the secondary channel [80, 107], the path of the C37 and C53 polypeptides to the catalytic center may be via a more frontal approach perhaps through the DNA cleft region (Fig. X) [91] although this remains to be determined.

Physical proximity data indicate that the association of the C37 C-region with the RNA:DNA hybrid binding region of C2 is more robust in the elongation complex than in the preinitiation complex [94]. Proximity of the C37 C-region to the β DloopII of C2 was also increased in the elongation complex. The corresponding loop in the pol II model is part of 'fraying site II' which interacts with the frayed, i.e., non-annealed, 3' terminal nucleotide of the RNA [125]. Bioinformatics suggested a possible structural homology between C53 and MLE, an RNA helicase [27]. Since C53 can interact with the 3' end of the RNA in the elongation complex the data suggest that it may participate in RNA:DNA hybrid melting thereby contributing to destabilization and termination [95].

Finally it should be noted that while lack of C37/53 leads to an increased elongation rate it does not render pol III Δ completely incapable of recognition of the SUP4 tRNA bipartite gene terminator [88]. This suggests that there is a C37/53/11-independent mode of termination intrinsic to the core pol III enzyme that is enhanced by C53/37 (A.A. & R.M., in preparation).

C11 involvement in intrinsic RNA 3' cleavage, termination and facilitated recycling

As first reported, C11 was believed to be directly involved in termination because [25] it was not known at that time that C11 mediates association of C53/37 with pol III [88]. C11 is a short polypeptide of 110 aa first identified as having strong homology to the pol II elongation factor TFIIS and responsible for the previously recognized robust intrinsic transcript cleavage activity of pol III [25, 82]. It has two Zn ribbon motifs separated by what may be a flexible linker. The N terminal Zn ribbon of C11 is homologous to the N terminal Zn ribbon of subunit RPB9 of pol II and the C terminal Zn ribbon is highly homologous to the Zn ribbon of the pol II RNA 3' cleavage factor, TFIIS [25]. Similar to TFIIS, the C11 Zn ribbon has the same two acidic residues at the tip of its loop that presumably position Mg²⁺ within the pol III catalytic center similar to the homologous residues of TFIIS in pol II [80, 107]. The N terminal domain of RPB9 is attached to the lobe domain of second largest subunit RPB2 forming a structure called the jaw of the polymerase [126]. As noted, the Rpb9-homologous domain of C11 (yellow in Fig. 2B) occupies a similar position on the C2 lobe of pol III and is required for association of C53/37 with pol III [28, 88, 91, 94].

Mutations to the C- and N-terminal motifs of C11 have different effects on termination; RNA 3' oligo(U) nibbling and prevention of terminator read through [26, 29]. Although the latter may be due to allosteric effects on C53/37 the cumulative data nonetheless provide clear evidence that C11 is positioned to affect pol III termination [25, 29, 88]. As detailed below, the RNA 3' cleavage activity of C11 is active during termination [26, 29] although the extent of its role in the termination process is unresolved. A challenge is to understand how the two domains of C11 work with each other and with C53/37 both at their surface locations and via their domains that approach the catalytic center of pol III, during termination.

3' terminal U residues are preferred substrates of C11-mediated cleavage by pol III [82, 84]. An enticing model would be that shortening of the rU:dA hybrid by cleavage-mediated removal of 3' terminal or frayed Us would lead to hybrid shortening, weakening and destabilization of the complex. However, this model was not supported by mutations in C11 that compromise RNA cleavage activity since although these mutations do lead to increased length of the 3' oligo(U) tract of the nascent RNA by 1–2 nts both *in vivo* and *in vitro*, this was not accompanied by increased terminator read through [26, 29]. The data suggest that the cleavage activity of C11 mediates 3' U cleavage during termination but not termination efficiency per se.

In striking contrast to the C-terminal mutants, a cluster of N-terminal C11 mutants exhibited increased terminator read through but not 3' oligo(U) lengthening [29]. The N-terminal mutations of C11 may affect termination via effects on C53/37 [29].

Far more C11 C-terminal domain mutants were isolated that impaired RNA cleavage than were N-terminal domain mutants that impaired terminator recognition, despite their isolation from the same library [26, 29]. Using the same screening approach applied to C37, the opposite was true; far more C37 mutants were isolated that impaired terminator recognition than 3' oligo(U) lengthening [30]. The same approach yielded even fewer C53 mutants using either both the terminator recognition and cleavage-sensitive screens [30]. These results provide evidence that of the C11, C53 and C37 subunits, terminator recognition was by far most sensitive to C37 [30].

It is noteworthy that C11 exhibits cleavage-dependent and cleavage-independent activities in pol III termination and associated processes. While C11 is required for facilitated recycling along with C53/37, a point mutant that disables its cleavage activity is nonetheless as active as the wild type C11 for facilitated recycling [88].

EXTRANEIOUS FACTORS MAY AFFECT POL III TERMINATION

Several trans-acting factors were shown to augment pol III termination *in vitro*. Among them La protein ranks first by historical perspective and because of its appealing specificity, affinity for the 3' oligo(U) tracts of pol III-terminated transcripts.

La exhibits sequence- and length-specific binding to oligo(U), with the 3'-OH group contributing substantially [127]. These features along with early findings that La is physically associated with all newly synthesized pol III transcripts *in vivo* made it a likely candidate for involvement in the termination process [127–131]. Experimental data obtained from *in vitro* assays supported that La is a transcription termination, transcript release and reinitiation factor for pol III [40, 48, 77, 118, 120, 132, 133], although such activities have been widely contested [134–136]. Only the non-phosphorylated fraction of human La was transcriptionally active *in vitro* while the phosphorylated fraction was inactive [120]. Consistent with this, non-phosphorylated La is associated with pol III transcribed genes *in vivo* while phosphorylated La is not [137]. Yet phosphorylated La is found associated with

nascent pre-tRNAs and other pol III transcripts in HeLa cells whereas non-phosphorylated La was not [138]. Although *in vivo* deletion of the La-homologous protein (Lhp1) from *S. cerevisiae* caused no decrease in pol III transcription levels [139], it led to increased accumulation of pol III on 5S rRNA genes *in vivo*, consistent with a role as a limiting factor for pol III termination [140]. Thus, although by 3' oligo(U) binding La protein provides a link between termination by pol III and processing of its transcripts, a role in the termination process per se and potential effects on recycling remain questionable. The mechanistic details of the link between pol III termination and RNA processing, and its implications, are described in a later section.

Other factors reported to promote pol III termination

These include transcription factor IIIC, topoisomerase-1 and PC4. Both topo-1 and PC4 copurify with TFIIC and enhance the TFIIC footprint on the downstream promoter [121]. Yeast TFIIC subunit TFC6 can be cross-linked to the terminators of tRNA and 5S rRNA genes suggesting a role in termination [141, 142]. In mammalian systems a TFIIC-associated activity caused a specific footprint on the terminator [143, 144]. Genetic mutagenesis-based screens of *S. pombe* that yielded C11 and C37 mutants that impair terminator recognition and/or 3' oligo(U) length [26, 29, 30], yielded no mutants for similarly-mutagenized *sfc6⁺*, the *S. pombe* homolog of TFC6 and mammalian TFIIC β [145], (K. R. and R. J. M., unpublished observation). Thus, to date, there is no data linking TFC6 or its counterparts to a functional role in termination.

The intriguing means by which NF1 promotes pol III termination of VA1 gene transcription may provide a clue into a mechanism by which some extraneous factors may operate [44]. Effects of NF1 on VA1 appear to be gene specific because a NF1 sequence-specific binding site is found adjacent to the pol III terminator of the VA1 but not other pol III-transcribed genes [44]. Consistent with this, ChIP analysis did not show preferred localization of NF1 to pol III transcribed genes [137]. It is plausible that NF1 bound to terminator-adjacent DNA acts as a roadblock that induces pausing at the terminator thereby promoting termination. Such a roadblock may also alter pol III confirmation by allosteric transmission upon collision. Consistent with this, a roadblock caused by a peptide nucleic acid was shown to increase termination even from a suboptimal terminator [146].

A role for chromatin in pol III termination

Although several studies show active involvement of chromatin in the control of pol III transcription [147–151], little is known about a role in termination. tRNA genes are generally nucleosome free [152]. Genome-wide ChIP that focused on the yeast histone variant H2A.Z revealed a large number of tRNA genes flanked by nucleosomes [153]. A recent study revealed that the dynamics of a nucleosome that abuts the terminator of the SUP4 tRNA gene in *S. cerevisiae* can modulate the expression level of the tRNA under different conditions [154]. Genome-wide pol III ChIP showed a significant fraction of pol III accumulation just beyond the terminators of tRNA and other class III genes, which may reflect pausing at a downstream nucleosome [50], similar to pol II accumulation downstream of its transcribed genes [155]. Accordingly, it seems possible that nucleosomes abutting terminators could increase pausing and thereby modulate termination and/or pol III release and reinitiation.

COUPLING OF POL III TERMINATION WITH RNA PROCESSING

As noted above, data indicate that the C11-mediated RNA 3' cleavage that occurs during termination is responsible, at least in part, for the variable lengths of the oligo(U) termini of several nascent pol III transcripts [Recently reviewed in 77]. The 1–2 nucleotide increase in

3' oligo(U) length can have striking effects on pre-tRNA turnover and maturation *in vivo* [26]. The subtle increase in 3' oligo(U) length that occurs in C11 cleavage mutants significantly increases the affinity of the transcripts for La protein with consequent protection from transcript degradation, more efficient RNA processing and increased levels of mature functional tRNA [26]. In the absence of La binding pre-tRNAs may succumb to nuclear surveillance-mediated decay by the exosome [156]. Indeed La levels are limiting for tRNA maturation in *S. pombe* but increasing La by ectopic expression or increasing the 3' oligo(U) length on pre-tRNAs in C11 cleavage mutants drives more nascent transcripts into the La-dependent productive pathway of tRNA maturation [26]. These observations suggest a role for termination-associated C11-mediated transcript cleavage in affecting functional tRNA production and raises the possibility that termination-associated nibbling by C11 helps control tRNA levels and thus translation. Consistent with this possibility is that a genome-wide search for candidate factors regulated by upstream open reading frames (uORFs) similar to uORFs that regulate GCN4 under general translational stress, revealed C11 as one of just a few *S. cerevisiae* genes [157].

While La has been found to transiently bind to all of the different types of nascent pol III transcripts examined, and its effects are most extensively characterized for tRNAs [reviewed in 77], it is also functionally involved in U6 snRNA maturation [158, 159]. As detailed below this may reflect a conserved mechanistic coupling between pol III transcription termination and U6 RNA 3' processing which involves an intricate series of what appear to be 3' uridylate-specific activities. Mature U6 snRNA has a 2'-3' cyclic phospho UMP residue at the 3' end, formed as part of a deuridylation-urydylate reaction [160]. The sequential terminal Us on pre-U6 snRNA that are formed as a result of pol III termination may be required for this process. La is a functional component of U6 3' end metabolism [158, 159].

Curiously, U6 snRNA is transcribed in all species by pol III while the other spliceosomal RNAs (U1, U2, U4, U5) are transcribed by pol II. However, unlike tRNA and 5S, the U6 snRNA sequence apparently can not accommodate an internal B box promoter element. Different species have developed varying mechanisms to circumvent the problematic need for a B box sequence in functional U6 snRNA genes. *S. cerevisiae* has the B box downstream of the terminator [161]; binding of TFIIC to the A and B box elements is facilitated by a positioned nucleosome between them [147, 149]. An alternative solution was achieved by *S. pombe*, in which the B box resides in an intron in the U6 gene that is spliced out upon maturation of U6 snRNA [162]. Vertebrates use yet another strategy; entirely upstream promoters for pol III-mediated U6 transcription [136]. In vertebrates this additionally involved the emergence of a Brf1-homologous protein known as Brf2 [136]. These observations suggest that U6 snRNA production can accommodate a variety of promoter types and positions which in some cases employ specially positioned nucleosomes, intron insertion, and factor-specific modes of initiation by pol III. These examples suggest that it is important that the U6 snRNA is a pol III transcript in these species and that the intricate uridylate-specific 3' end processing accounts for its dependence on pol III.

The coupling of a pol III termination mechanism that produces RNAs with 3' oligo(U) ends that mediate specific association with the La protein maturation factor is undoubtedly a means to afford pol III transcripts a processing pathway separate from the pols I & II transcript maturation pathways.

It was recently reported that pol III transcription and precursor tRNA processing are linked via a pathway that involves the pol III repressor Maf1 although this is likely due to saturation of processing and/or export machinery [163]. A role for RNA'se P in coupling pol III transcription and tRNA 5' processing has been proposed [164, 165], whereas all other

links between the transcription and processing of class III genes appears to be via 3' end processing as described above.

CONCLUDING REMARKS

Transcription termination involves pausing followed by destabilization of the elongation complex with dissociation of its components. Bacterial RNA polymerase as well as pol II can terminate transcription in response to more than one terminal signal. Pol II termination of poly(A)-containing mRNA synthesis uses a cis-acting terminator element, the poly(A) addition site, that acts at a distance to initiate the process, followed by dissociation of the complex somewhere downstream, somewhat similar to the Rho-dependent mechanism of *E. coli* transcription termination. Pol III can achieve the same outcome on a short oligo(dA) template that serves to both pause and dissociate the complex with efficiency and near nucleotide precision, somewhat similar to the intrinsic termination by *E. coli* RNA polymerase. Yet, despite the efficiency of what appears to be a simple termination signal for pol III, involvement of multiple subunits suggests a complex mechanism of termination. Recent reports of termination defective mutants of C37 and C11, and localization of these plus C53 near the catalytic site suggest active alteration of the pol III active center during termination, as opposed to polymerase jaw-mediated brakes clamping down on incoming DNA and passive cessation of RNA synthesis. Control of elongation rate as well as the degree to which the oligo(rU:dA) hybrid confers instability to the pol III complex are likely underlying components of the termination mechanism that are sensitive to the activities of the termination subunits. Because C37, C53 and C11 have homologs in the pols I & II systems it is suggested that these polymerases may use similar mechanisms to execute their final stages of termination, complex destabilization, dissociation and release.

High-resolution crystal structures of pol III elongation complexes with and without C11/C53 and C11 will undoubtedly reveal more about these subunits and their involvement in the catalytic center. Another challenge will be to obtain crystals of a pol III termination complex.

An aspect that we believe will provide unique insight into the mechanisms of pol III termination is to understand the species-specific differences in the minimal number of Ts required, as well as oligo(dT) context-dependent effects, and how terminator-adjacent binding sites for DNA-binding factors may promote termination. In addition, 'secondary terminators' and non-canonical pol III terminators are also a potential trove of important means to production of a newly emerging class of tRNA-downstream short RNAs.

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HIGHLIGHTS

- The rU:dA(5–6) hybrid is a destabilizing component of transcription termination.
- Pals I, II & III initiate termination differently but may share features thereafter.
- Several pol III subunits, including C2, C11, C53 and C37 contribute to termination.
- C37/53 and C11 are homologous to TFIIIF α / β , respectively.
- Extraneous factors may be recruited to affect pol III termination in some cases.

Figure 1. Schematic of termination mechanisms by multisubunit RNA polymerases

The mechanisms reveal three themes common to more than one pol, i) 5'-3' exonuclease digestion of a downstream fragment of the nascent transcript attached to the polymerase, ii) weakly base pairing oligo(rU:dA) hybrid and iii) involvement of a helicase, Rho or Sen1. **A)** Rho mediated termination by bacterial RNA polymerase. Rho helicase binds to the nascent transcript at a C rich region and travels along the RNA in a 5' to 3' direction toward the polymerase to induce destabilization and termination. **B)** Intrinsic termination by bacterial RNA polymerase. A hairpin formed by the nascent RNA followed by a T-rich stretch on non-template strand comprise the intrinsic termination signal. Transcript release occurs within the T stretch. **C)** Pol II termination mechanism for poly(A)-containing mRNA-coding genes. Transcript is cleaved downstream of the poly(A) addition site by cleavage factors associated with the pol II CTD. The polymerase-attached RNA fragment is digested by Rat1/Xrn2 in 5'-3' direction. Sen1 helicase also binds to pol II and is required for termination. This figure is over simplified to emphasize similarities with other polys. Also, there are different pathways for termination at different classes of genes (see text). **D)** Eukaryotic pol I termination mechanism. Most of the transcripts are terminated upstream of the Nsi1 (Ydr026C) binding site within the T stretch. Cotranscriptional cleavage of the transcript followed by processive 5'-3' cleavage by Rat1 is also involved in termination. **E)**

Pol III termination mechanism. A stretch of Ts on the non-template strand is sufficient to direct termination.

Figure 2. RNA polymerase III subunits and structural elements that affect termination

A) Comparison of subunit compositions of pols II and III. The termination subcomplex that shows homology to TFIIF α/β subunits, the initiation subcomplex that shows similarity to TFIIE α/β , and C11 which is homologous to RPB9 at its N terminus and to the C-terminal motif of TFIIS at its C terminus, are highlighted. **B)** Cartoon of surface features of pol III derived from the electron microscopic structure of pol III (EMD-1802) deposited by [91]. Blue color reflects the proposed position of the C53/37 dimerization domains and pink shows the initiation subcomplex [91]. The cartoon of the RNA:DNA template hybrid was obtained from pol II elongation structure (PDB ID: 3HOV). The non-template strand is not shown. Yellow color reflects localization of the N-terminal, Rpb9-homologous domain of C11 only. Other features discussed in the text and/or elsewhere in the figure are indicated. For other structural representations, refer to [91]; for additional models see figures 3 & 5 in [94]. **C)** Cartoon depicting the structural elements that are involved in transcription elongation and termination. The catalytic site along with the hybrid binding region and other elements such as the fork loops, the trigger loop and bridge helix are considered as part of a larger, extended active center.