COMMENTARY

Changing directions in Pol III transcription

Approximately 8 years ago, our thinking about the organization of promoter elements in DNA took a sudden turn. The realization that initiation of transcription by RNA polymerase III relied upon intragenic sequences, and thus differed significantly from the paradigm then provided by prokaryotic RNA polymerases, raised many questions about the origins of the various transcription systems and why they might be so different.

These questions are slowly being answered. Comparisons of the structures of RNA polymerases of eukaryotes and of prokaryotes are revealing their conserved functional elements, indicating they have common origins (for review, see Buhler et al. 1987). Concurrently, some of the ostensibly major differences between eukaryotic RNA polymerase II promoter elements and prokaryotic promoters (notably, the importance of far upstream sequences) no longer appear to be that distinctive (see Ptashne 1986). And now, an ever-increasing volume of information is dispelling the notion that promoter elements recognized by any of the eukaryotic RNA polymerases are necessarily unique.

In the February issue of *Genes & Development*, two articles provide additional detail to what is known about the mechanism of RNA polymerase III transcription initiation, and raise interesting questions about whether the control of such transcription might not resemble that utilized by RNA polymerase II. Kunkel and Pederson (1988) report that transcription in vitro of human U6 snRNA by an RNA polymerase III-like enzyme is dependent upon sequence elements 43–67 nucleotides upstream of the transcription start site. Furthermore, they report that transcription in vivo relies upon distal 5'-flanking sequences, including a region containing the well-known 'octamer' motif (ATGCAAAT) that is required for expression of immunoglobulin, histone, and U2 snRNA genes (see Fletcher et al. 1987 for a recent discussion of the importance of this motif and its associated binding protein). Kunkel and Pederson also report that the U2 snRNA gene promoter elements (which are utilized by RNA polymerase II) can in some instance replace the naturally occurring U6 snRNA gene upstream elements.

These data follow on the heels of experiments reported by Bark et al. (1987) and by Carbon et al. (1987). The latter group reports that the *Xenopus tropicalis* U6 snRNA gene relies upon at least three 5'-flanking elements for transcription in vivo. Two of these are the same as the human U6 snRNA proximal and distal elements studied by Kunkel and Pederson, and the third is a TATA motif close to the transcription initiation start site. These three motifs are present in all known U6 snRNA genes and remarkably resemble promoter elements utilized by RNA polymerase II.

The U6 snRNA genes also contain what may be a 'canonical' RNA polymerase III box A promoter element, previously shown to be important for the transcription of tRNA, adenovirus VA, and 5S RNA genes (reviewed in Sharp et al. 1985). Remarkably, neither this element nor, for that matter, are any of the gene's internal sequences required for transcription in vitro (Carbon et al. 1987; Das et al. 1988). Only when placed in competition with other RNA Pol III transcribed genes does the box A internal sequence confer a transcriptional advantage to the U6 snRNA gene (Carbon et al. 1987). Thus, we do not yet know the extent to which U6 snRNA transcription in vivo relies solely upon upstream elements, nor are there insights into whether any of these elements participate in the regulation of transcription.

Findings analogous to these in several respects have been reported recently by groups studying transcription initiation of the human 7SK and 7SL RNA genes. As with the U6 snRNA genes, upstream sequences are required for transcription of both types of 7S RNA genes (Ullu and Weiner 1985; Murphy et al. 1987). The 7SK genes also can rely solely upon upstream sequences for transcription initiation in vitro. These upstream sequences include a TATA motif. Although it is by no means certain that this motif is a site of action of the RNA polymerase II transcription factor TFIID, its presence and significance for 7SK RNA transcription is provocative. The 7SK RNA genes have no obvious internal sequence homologies with tRNA, VA, or 5S RNA genes, but firm proof that internal sequences are unimportant for transcription in vivo has not yet been produced.

These studies of transcription initiation of the U6, 7SK, and 7SL RNA genes strongly implicate usage of upstream elements, some of which are common to RNA Pol II transcribed genes. However, caution is warranted before jumping to the conclusion that RNA polymerase II and RNA polymerase III can follow common paths to prospective initiation sites. The evidence that the U6, 7SK, and 7SL genes are transcribed by 'bona fide' RNA polymerase III relies solely upon α -amanitin insensitivity, the utilization of pol III termination signals, and competition assays with 5S RNA genes (Kunkel et al. 1986; Carbon et al. 1987; Reddy et al. 1987; Das et al. 1988). Studies with purified RNA polymerases and transcription factors, together with more extensive competition analyses, are required to establish solidly that it is the same RNA polymerase III holoenzyme that transcribes these genes as transcribes tRNA, VA, and 5S RNA genes.

Transcription of *Xenopus* 5S RNA genes requires RNA polymerase III, TFIIA, TFIIB, and one or more proteins collectively called TFIIC. TFIIA binds the 5S RNA gene internal control region and helps recruit

TFIIIB and TFIIIC to a stable transcription complex [Shastry et al. 1982; Lassar et al. 1983; Setzer and Brown 1985]. Heretofore, much attention has been paid to TFIIIA, largely because it was readily purified. It was the first eukaryotic transcription factor to be characterized, and it is the prototypical zinc-finger domain DNA binding protein [Rhodes and Klug 1987].

Following fertilization of *Xenopus* oocytes, a remarkable developmental program ensues. As part of this program, transcription of a vast excess of oocyte 5S RNA genes is repressed while transcription of a relatively small number of somatic 5S RNA genes is activated. The severalfold lower affinity of TFIIIA for a subclass of oocyte 5S RNA genes compared to the major somatic 5S RNA genes has been suggested to be the cause of the preferential inactivation of all the oocyte genes [Brown and Schlissel 1985]. It is now apparent that this explanation does not suffice, and additional factors must be invoked to explain the transcriptional switch.

In the February issue of *Genes & Development*, McConkey and Bogenhagen (1988) demonstrate that TFIIIA binds equally well to the *Xenopus* major class of oocyte 5S RNA genes as to the somatic 5S RNA genes. Thus, everything else being equal, the first step in the formation of a stable transcription complex, the association of TFIIIA with 5S DNA, should occur equally well on these genes in vivo. However, everything else does not appear to be equal, for in a transcription system derived from mature oocytes, the oocyte 5S RNA genes are transcribed in vitro at only a few percent of the rate of the somatic genes. These results confirm similar results recently reported by Peck et al. (1987) and by Millstein et al. (1987).

If a difference in TFIIIA affinity is not the cause of the transcriptional switch between somatic and oocyte genes, then what is? These genes differ from each other by a few nucleotide substitutions near the 5' end of the internal control region. McConkey and Bogenhagen point out that the pattern of TFIIIA-induced protection from DNase I cutting of the trace oocyte, major oocyte, and somatic 5S RNA genes differs near the 5' end of the internal control region, and suggest this might alter the association of TFIIIC, the second essential component in the stable transcription complex. Precedent for the importance of this part of the internal control region for binding of TFIIIC derives from an analysis of point mutants of the 5S RNA gene [Pieler et al. 1987]. However, Millstein et al. (1987) report that supplementation of oocyte extracts with TFIIIC appears not to increase the transcription of the oocyte genes which suggests these differences do not weaken TFIIIC binding. They might, however, affect the subsequent interaction of RNA polymerase III with the stable transcription complex. A more careful assay such as that devised by Pieler et al. (1987) is required to explore those questions.

Recent work by Wolffe and Brown (1987) further implicates the structure of the transcription complex, rather than its assembly, as being important for the selective expression of somatic 5S RNA genes. They observed that egg extracts as well as whole oocyte extracts

contain an activity that strips transcription complexes from oocyte 5S RNA genes. Presumably this activity (which fractionates with ribosomes through several steps) recognizes some aspect of the TFIIIC/TFIIIA/DNA structure assembled near the 5' end of the internal control region. How this activity succeeds in removing a complex that is exceedingly stable in vitro to high ionic strength [Setzer and Brown 1985; Carey et al. 1986; Jahn et al. 1987], and to the transit of multiple RNA polymerases [Wolffe et al. 1986] is certainly not clear.

Apart from the few base changes in the 5S RNA internal control region, the only sequence differences between the *Xenopus laevis* major somatic and oocyte 5S RNA genes occur in the spacer regions. Heretofore, these spacer regions have not been viewed to be of major significance, for in a germinal vesicle transcription system they have little effect upon transcription initiation [Wormington et al. 1981]. However, that upstream sequences can be important for 5S RNA transcription has been documented with genes from *Bombyx* and *Neurospora*. These, as well as the 5S RNA genes of *Diptera*, contain conserved upstream sequence elements resembling the RNA polymerase II TATA motif [Morton and Sprague 1984; Rubacha et al. 1984; Selker et al. 1986; Garcia et al. 1987]. Exploration of the importance of these sequences and of the spacer regions in *Xenopus* 5S RNA genes in appropriate transcription systems may shed new light upon the assembly of transcription complexes and the developmentally regulated expression of 5S RNA synthesis in *Xenopus*.

Perusal of these reports and of much accumulating evidence that tRNA synthesis is modulated, and in some instances absolutely dependent upon 5'-flanking sequences (I have not mentioned this evidence for lack of space), makes it clear that there is still much to be learned about the initiation of transcription by RNA polymerase III. This new information, although raising more questions than it answers, diminishes a seemingly major difference between the promoter elements utilized by RNA polymerase II and RNA polymerase III. It is now apparent that all three eukaryotic RNA polymerases utilize transcription complexes assembled at or near the site of transcription initiation, and can rely upon either upstream or downstream sequences for their formation. Furthermore, within the cell these complexes appear not to be as immutable as once believed. Thus, the differences between these transcription systems are less distinctive than first imagined. The next few years should lead to a greater understanding of how the different parts of the eukaryotic transcription apparatus are related, and to how and why those parts have evolved their distinctive features.

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