MILESTONE 8

It ain't over until the polymerase falls off

Today, it is widely accepted that RNA chain elongation and termination by RNA polymerase II (pol II) is a complex process that is coordinated with 3′-end processing and polyadenylation of the primary transcript. Just over 20 years ago, however, the identification of the first elongation factor, TFIIS or SII, provided the initial indication that RNA pol II transcription could be regulated at the level of elongation.

Back in 1973, Natori and colleagues identified SII by its ability to stimulate transcription *in vitro* and to enable pol II to synthesize long transcripts. Yet its mode of action remained unknown until 1992, when three groups added a considerable piece to the puzzle by providing insights into the mechanism of SII activity. Reines, and Izban and Luse, noticed that the addition of SII caused a shortening of transcripts associated with stalled RNA pol II. They found that, in the presence of SII, the RNA pol II complex can serve as a nuclease, cleaving its nascent transcript from the 3′ end. Wang and Hawley also presented evidence to support these observations, and proposed a possible proofreading role for the activity described. Surprisingly, it was noted that this process leaves the pol II complex intact and the remaining transcript can subsequently be elongated. The nuclease activity that is stimulated by SII helps pol II bypass specific blocks to elongation and therefore increases elongation efficiency.

A question that puzzled the community was how 3′-end processing was linked to termination. The connection between these processes was established when it became apparent that polyadenylation and transcription termination were dependent on the same DNA sequences at the 3′ ends of genes. A role for poly(A) site cleavage in termination was first established by two groups — Logan and colleagues, and Connelly and Manley. Based on the hypothesis that polyadenylation must be a prerequisite for RNA pol II termination, because this would ensure that mRNA-coding sequences were completely transcribed before a termination event occurred, they introduced several single-base-pair mutations into the polyadenylation motifs, and showed abrogation of both polyadenylation and termination.

Two models were proposed to explain these results. The first postulated that the emergence of polyadenylation sequences on the RNA triggers a change in the factors associated with the polymerase, which eventually results in termination. The second, also known as the 'torpedo' model, states that cleavage of the transcript is required to trigger termination. Recent evidence supports both models and, until the mystery is solved, only one thing is clear: it ain't over until pol II falls off.

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MILESTONE 9

An alternative string theory

The nucleosome hypothesis represents one of the great paradigm shifts in our understanding of eukaryotic gene expression. Formulated by Roger Kornberg in 1974, its key concept is that eukaryotic DNA is tightly packaged around a core of structural proteins — histones — to generate a nucleosome array that is fundamental for controlling gene expression.

Throughout the 1960s, it was largely accepted that chromatin was a linear strand of DNA coated with a simple repeated arrangement of five histones that packaged it into so-called '100-Å fibres'. This view held that the DNA is encased within the histone protein, resulting in non-specific repression of transcription.

However, cracks in this view were beginning to surface. There was accumulating evidence to indicate that chromatin structures might not be so evenly distributed as originally thought. Evidence from X-ray diffraction, electron microscopy, and, in particular, a 1973 study by

MILESTONE 10

Silent remembrance

Generally speaking, eukaryotic cells do not discard DNA as they differentiate. Cellular differentiation therefore has to be explained as the consequence of differential gene expression. So how are genes stably yet reversibly regulated? During the past 30 years, the direct modification of DNA by methylation has been shown to have a central role in repressing gene expression and transmitting the silenced state to daughter cells.

Among the founding papers in the field were the 1975 reviews by Arthur Riggs, and by Robin Holliday and John Pugh, who discussed the literature on DNA methylases in bacteria. Their models proposed that the properties of these enzymes — in particular, their preference for hemi-methylated substrates — made them ideally suited to establish stable differentiated states in the absence of genetic mutation. They further proposed that the sequence-specific binding of these enzymes would have a gene-regulatory role. This idea was not