Cell division

Coordinating histone transcription and DNA replication

from Paul Nurse

ONE of the more intriguing puzzles posed by the cell division cycle is how the various periodic processes required in the cycle are temporally cordinated. A good example is the coordination of histone supply with DNA replication, since in most eukaryotic cells histone protein synthesis appears to be confined to S phase[1]. This is true of the budding yeast *Saccharomyces cerevisiae*[2], and a series of recent papers from Hereford and her collaborators have provided considerable insight into how DNA replication and histone synthesis are coordinated in this organism.

Control at the level of transcription of the histone genes appears of major importance in maintaining coordination. This was established by using cultures synchronized with the yeast pheromone α-factor, which blocks cells in G_1, and cells fractionated by elutriation centrifugation according to size, and thus position in the cell cycle[3]. Monitoring the histone H2A and H2B transcripts by northern blot hybridization and the H3 and H4 transcripts by *in vitro* translation showed that all histone messages reached a peak level during S phase. That the periodic accumulation of histone messages is the result of increased histone gene transcription was demonstrated by pulse-labelling α-factor-synchronized cells and hybridizing the labelled RNA to H2B DNA immobilized on a filter[4]. The rate of H2B transcription peaks at the G_1–S phase boundary and can account for the peak in the level of H2B transcripts seen a little later in mid-S phase.

A second level of regulation operates post-transcriptionally. When DNA replication is blocked using a temperature-sensitive mutation in *cdc*8, a gene function required for DNA synthesis, then the turnover of H2A and H2B transcripts rapidly increases[3]. This produces a rapid fall in the levels of histones, as has been shown when DNA replication is blocked using hydroxyurea[2]. The results suggest that there are two major controls which coordinate histone supply with DNA replication. First, there is an activation of histone gene transcription at the G_1–S phase boundary, and second, there is a stabilization of histone messages by the process of DNA replication itself. The two controls would result in a sharp increase in the amount of message at the beginning of S phase, followed by a rapid drop at the end of S phase when DNA replication ceases and turnover of histone messages consequently increases.

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Two elegant papers from Hereford's laboratory have given more information about the mechanism of activation of histone gene transcription. In one paper, the point in the cell cycle at which transcription is activated has been defined precisely using a *cdc*7 temperature-sensitive mutant which blocks cells in late G_1 (ref.4). Cells synchronized with α-factor and then arrested at the *cdc*7 block point show an increase in the rate of H2B transcription even though DNA replication is not initiated. It is thus traverse through a late stage of G_1 which activates histone transcription, rather than the onset of S phase. Furthermore, in the cells arrested at the *cdc*7 block point the rate of H2B transcription remains at a high level, suggesting that traverse of a later stage in the cell cycle is required to turn transcription off. This stage must be before the mid-S phase block point imposed by a *cdc*8 temperature-sensitive mutant since in these arrested cells the rate of H2B transcription shows the normal pattern of a peak followed by a fall to a low level. This behaviour would be expected if histone transcription ends with the first third of S phase.

In the second paper, a DNA sequence next to the H2A and H2B genes is shown to be necessary for periodic transcription during the cell cycle[5]. The H2A and H2B genes are adjacent, but separated by a spacer region of about 800 nucleotides in which transcription of both genes is initiated. First, the H2A and *lac*Z genes were fused to construct a gene coding for a hybrid protein made up of the first part of H2A histone and β-galalctosidase, and under H2A transcriptional control. A plasmid containing the H2A–*lac*Z fusion gene along with the 800 nucleotide spacer region and part of the H2B gene was then integrated into the chromosome at the site of the *leu*2 gene. Cells synchronized with α-factor and containing the integrated plasmid synthesized β-galactosidase at a low level but synthesis did not change periodically during the cell cycle. When the experiment was repeated with a plasmid containing an extra 2.3-kilobase fragment encompassing the rest of the H2B gene and some sequences flanking its 3′ end β-galactosidase was synthesized at a higher level and periodic variations were seen with peak activity at the same time that H2B transcripts peak in amount. Clearly a DNA sequence located at the 3′ end of the H2B gene is required for regulation of H2A gene transcription in the cell cycle.

The same sequences also contain an *ars* (autonomous replicating sequence) activity, the presence of which enables the plasmid to transform yeast cells at high frequency. Deletion mapping has shown that *ars* activity and the effects on β-galactosidase synthesis must be within 50–100 nucleotides of one another. As *ars*-containing plasmids can replicate in the yeast cell the *ars* sequences would seem to contain a chromosomal origin of replication[6]; if so, then the sequence required for periodic histone transcription might also be a replication origin. Osley and Hereford suggest that the changes in chromatin structure that are required to initiate DNA replication at the beginning of S phase also activate histone transcription, and that activation of the replication origin once every cycle could be responsible for the periodic synthesis of histone mRNA.

Although the identification of *ars* sequences with replication origins remains uncertain, this does not affect the main conclusion that a particular sequence is required for periodic histone transcription, and that the sequence can respond to the changes in chromatin structure that take place when yeast cells traverse the cell cycle. Folded chromosomes (similar to nuclear matrix preparations only isolated in low salt) sediment differently in cells blocked at different stages of G_1, and at stages from G_1 through S phase to G_2 (ref.7), and there are also alterations in the association of plasmid with folded chromosomes which occur early during the cell cycle[8]. Some general change obviously must occur in chromatin state in late G_1 — perhaps a gradual decondensation or an altered binding to nuclear matrix — and could influence the histone-associated *ars* so that it activates histone gene transcription.

Such a control mechanism may be used quite generally to regulate expression of genes whose products are required in late G_1 or S phase; indeed, there is some preliminary evidence indicating that the transcription of the HO gene involved in mating-type switching may be regulated in a way similar to the histone genes (K. Nasmyth *Nature*, in the press). It is also possible that the same control mechanism is used in other eukaryotic cells since in most cell types histone gene transcription usually only occurs during S phase, and there are considerable changes in chromatin structure as cells traverse G_1 and initiate DNA replication[1]. Monitoring the behaviour of the *S. cerevisiae* histone gene fusions in other eukaryotic cells will make it possible to test this possibility. □

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