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M. L. Birnstiel

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Sea urchin histone genes: the beginning and end of the message

By M. L. Birnstiel
Institut für Molekularbiologie II der Universität Zürich, Hönggerberg, 8093 Zürich, Switzerland

This is a brief summary of the talk given at the Meeting.

The purpose of studies on the regulation of histone gene expression is to explain, for instance, how histone proteins arise in defined stoichiometric relationships in the chromatin, how transcription of histone genes is regulated in the cell cycle and how during the development of some species, histone variant genes are activated sequentially. The control of histone gene expression has many interesting facets. One is struck by the major differences in balance and importance of the various regulatory mechanisms as they become apparent from investigations in many laboratories. For example, in yeast, histone gene transcription is tightly coupled to the cell cycle, and the amounts of histone synthesized are determined largely by regulation of histone mRNA turnover (Hereford & Osley 1981). At the other extreme, there is the example of the maturing frog oöcyte where histone mRNA synthesis is uncoupled from DNA synthesis and yields pools of histone 1000-fold in excess of nuclear DNA mass (reviewed by Woodland 1980). Recent reports suggest that even the details of histone gene transcription may vary during the development of the species. The tandem histone gene clusters of sea urchin (G. Spinelli, unpublished results) and frog oöcytes are transcribed polycistronically at least at some stages of their development (J. Gall, personal communication), whereas histone gene clusters of the cleaving sea urchin embryos appear to be transcribed monocistronically (Mauron *et al.* 1981). Finally, in the early embryo the partitioning of the mRNA between nucleus and cytoplasm may be also a regulative process (DeLeon *et al.* 1983).

Regulatory sequences in and around histone genes of the sea urchins have been identified and analysed in our laboratory (Grosschedl *et al.* 1983; Grosschedl & Birnstiel 1982; Grosschedl & Birnstiel 1980*a*, *b*; Grosschedl *et al.* 1981; Birchmeier *et al.* 1982; Birchmeier *et al.* 1984). This was done by *in vitro* mutation of the DNA sequences of cloned histone genes. The biological effects of such sequence manipulation were then determined by inserting wild-type and mutated genes into the frog oöcyte nucleus (Mertz & Gurdon 1977; Probst *et al.* 1979; Kressmann *et al.* 1978; Kressmann & Birnstiel 1980) and comparing the rate with which histone mRNA was synthesized. Such investigations have provided some answers as to the genetics of gene control.

Frog oöcytes show certain lesions in transcription of sea urchin histone genes and RNA processing, which can be overcome by co-injection of chromosomal salt wash fraction obtained from sea urchin embryos (Stunnenberg & Birnstiel 1982; Galli *et al.* 1983; Birchmeier *et al.* 1984). A biochemical complementation test is thus established which allows us to identify and purify components that are absent in frog oöcytes but present in the sea urchin embryos, and by this procedure to characterize essential components of gene transcription (Jan Mous & Hendrik Stunnenberg, unpublished results) and RNA processing (Birchmeier *et al.* 1984). This general approach has given us new insights into the biochemistry of gene control.

Transcription of the sea urchin histone genes in the frog oöcyte nuclei is sluggish, but can be considerably enhanced in the case of the H2B histone gene when chromosomal components eluted at 0.3 M NaCl are co-injected. Fractionation by heparin sepharose, DEAE-sephacel and CM-sepharose column chromatography enriches an active component with a molecular mass of approximately 50 kDa (Jan Mous & Hendrik Stunnenberg, unpublished results), which can be assayed by oöcyte injection experiments.

By using the DEAE sephacel fraction, we have attempted to locate potential sites of interaction for this stimulatory factor. Deletion of the CAAT, the H2B specific box or the TATA motif all create down mutations (Jan Mous & Hendrik Stunnenberg, unpublished results), but these sequences are apparently not major address sites for the stimulatory factor. Rather, the coding body of the gene itself appears to be implicated. This can be demonstrated by transferring the mRNA coding portion of the H2B gene to the H2A promoter: such a chimeric gene can now be seen to be expressed under control of the H2B stimulatory factor (Jan Mous & Hendrik Stunnenberg, unpublished results).

The DNA sequences giving rise to the 3′ termini of histone mRNAs are flanked by conserved sequence blocks, a hyphenated inverted DNA repeat being situated immediately upstream, a CAAGAAAGA sequence 8 b.p. downstream (Busslinger *et al.* 1979). Both these sequences are essential elements for the generation of faithful 3′ ends of histone mRNA (Birchmeier *et al.* 1982; Birchmeier *et al.* 1983) and intervene at the level of the RNA sequences during RNA processing of the precursor (Birchmeier *et al.* 1984).

It is now accepted that the 3′ termini of mRNAs, including those of histone mRNAs, are, or at least can, be generated by RNA processing. For histone mRNAs this has been deduced from a series of experiments in which *in vitro* synthesized histone mRNA precursor molecules were injected into the frog oöcyte nucleus (Birchmeier *et al.* 1984; Krieg & Melton 1984). It was thus demonstrated that precursors with 3′ extensions were processed to yield faithful 3′ ends of histone mRNA. Maturation of histone mRNA precursors has now been shown to occur for transcripts of a variety of sea urchin histone genes (Birchmeier *et al.* 1984; Fred Schaufele, unpublished results), the H2B gene of the chick (Krieg & Melton 1984), the H4 gene of *Xenopus laevis* (Oleg Georgiev, unpublished results). In fact, it occurs for all histone mRNA precursors tested so far, with the sole exception of the H3 gene transcripts of the sea urchin *Psammechinus miliaris* where this process is very inefficient (Hentschel *et al.* 1980). In this case processing requires the complementation of the frog oöcyte with a 60 nucleotide RNA species of the sea urchin (Galli *et al.* 1983; Birchmeier *et al.* 1984) which is a component of a small nuclear RNP.

We have recently studied the mode and kinetics of RNA processing for *in vitro* synthesized histone mRNA precursors of a series of histone genes of the sea urchin and of an H4 gene of *Xenopus laevis*. We have preliminary evidence that 5′ capping of the transcript is a prerequisite for 3′ processing of the RNA. We find that the homologous H4 mRNA accumulates within minutes after injection of *in vitro* synthesized precursors into the oöcyte. 3′ processed H4 mRNA molecules exit into the cytoplasm with a time lag of about 30 min. RNA migration does not, therefore, appear to be coupled directly with RNA maturation.

In summary, the DNA sequences preceding the cap sites of sea urchin histone H_2B mRNA gene contains highly conserved sequence motifs which can be shown by deletion to modulate the rate with which histone mRNAs are produced in the frog oöcyte nucleus. Among these upstream sequences, the TATA box is exceptional in that it not only influences the rate of transcription but also determines the position of the first nucleotide of the mRNA. We have

now identified a DNA segment within the mRNA coding body of the H2B gene which appears implicated as a site of action for the H2B stimulatory factor. Several elements near the 3′ terminus of the gene are required for the generation of 3′ ends by RNA processing: the conserved sequences flanking the 3′ terminus of the mature mRNA; the spacer sequences immediately following; 5′ capping of the precursor RNA; and, finally, a 60 nucleotide RNA which presumably resides within a small nuclear RNP.

Thus, the complex regulatory features of histone gene expression appear to be based on a plurality of sequences lying 5′, 3′ as well as internal to the gene. We also demonstrated an editing system in which the 3′ processing of histone mRNA precursors is mandatorily coupled with 5′ capping of the message and, additionally requires the intervention of a small nuclear RNP. These newly discovered features of histone gene expression and RNA processing greatly increase the potential number of points at which regulation of gene expression can occur.

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