Two *Drosophila* chorion genes terminate transcription in discrete regions near their poly(A) sites

Y.N.Osheim, O.L.Miller, Jr¹ and A.L.Beyer

Department of Microbiology and ¹Department of Biology, University of Virginia, Charlottesville, VA 22908, USA

Communicated by B.Daneholt

We have examined transcription termination of two closely linked *Drosophila melanogaster* chorion genes, *s36-1* and *s38-1*, using the electron microscope. Our method is unusual and is independent of *in vitro* nuclear run-on transcription. By measuring transcription unit lengths in chromatin spreads, we can localize efficient termination sites to a region of ~210 bp for *s36-1* and ~365 bp for *s38-1*. The center of this region is ~105 nucleotides downstream of the poly(A) site for the *s36-1* gene, and ~400 nucleotides downstream for the *s38-1* gene. Thus, these two *Drosophila* chorion genes terminate more closely to their poly(A) addition sites and in a shorter region than many other polyadenylated genes examined to date.

Key words: transcription termination/*Drosophila* chorion genes/electron microscopy/chromatin spreads/transcription unit lengths

Introduction

Little is known about the transcription termination process in polyadenylated genes transcribed by RNA polymerase II (reviewed by Birnstiel *et al.*, 1985). In all cases examined, except possibly yeast, transcription continues beyond the poly(A) addition site, often for a 1000 or more nucleotides (reviewed by Nevins, 1983). In several cases, termination appears to occur at multiple, weak, ill-defined sites (Citron *et al.*, 1984; Hagenbuchle *et al.*, 1984; Rohrbaugh *et al.*, 1985). The primary transcripts are cleaved and polyadenylated at discrete sites to generate the 3' end of the mature mRNA species (e.g. Ford and Hsu, 1978; Nevins and Darnell, 1978; reviewed by Nevins, 1983 and Birnstiel *et al.*, 1985).

We have utilized the electron microscope (EM) to analyze transcription termination of two highly transcribed *Drosophila* chorion genes. The *s36-1* and *s38-1* genes (hereafter referred to as *s36* and *s38*) code for two major chorion (eggshell) proteins in the follicle cells of *D. melanogaster* females (Petri *et al.*, 1976; Waring and Mahowald, 1979). The chromosomal region containing the genes is preferentially amplified during the later stages of oogenesis (Spradling and Mahowald, 1980; Spradling, 1981; Orr *et al.*, 1984). Shortly after amplification is initiated, the genes become transcriptionally active (Orr *et al.*, 1984; Spradling and Mahowald, 1979). *s36* and *s38* are short, prolifically transcribed genes separated by a 1416-bp spacer (Figure 1a). The genes and spacer have been sequenced (B.Wakimoto, J.Levine and A.Spradling, personal communication). Because of their stage-specific expression, amplification and linkage, the genes are readily identified when appropriately-staged follicle cell chromatin is prepared for EM visualization (Osheim and Miller, 1983). This has allowed us the unique opportunity to map the transcription termination sites of two chorion genes directly from the electron-micrographs.

Results and discussion

Figure 1a is a map of the two linked chorion genes based on the DNA sequence of B.Wakimoto *et al.* (personal communication). The gene lengths of 1100 bp for *s36* and 1516 bp for *s38* refer to the distances from the transcription initiation sites to the poly(A) addition sites. A representative *s36*–*s38* gene pair, as visualized in the EM, is shown in Figure 1b, and micrographs of several others have been published (Osheim and Miller, 1983; Osheim *et al.*, 1985). Both the *s36* and *s38* transcription units exhibit a fairly uniform and high transcript density with a single gradient of transcript length; thus the terminal transcripts on each unit are full length. We argue that the abrupt transition from transcribed gene to transcript-free spacer represents termination rather than cleavage of the nascent RNA, with termination being characterized by disengagement of the polymerase and release of the RNA transcript. Cleavage is ultrastructurally distinguishable in these EM spreads. As previously reported, one can see polymerases on the template either devoid of visible transcripts (Trendelenburg, 1982) or with shorter-than-expected transcripts (Laird and Chooi, 1976; Beyer *et al.*, 1981) if cleavage has occurred. RNA polymerase molecules are larger, more electron-dense, and easily distinguishable from nucleosomes in these preparations (Martin *et al.*, 1980). We do not observe polymerase molecules devoid of transcripts at the immediate end of the transcription units, or transcripts in the inter-gene spacer or downstream of *s38* for a distance of at least 1 kb. We proceed on the assumption of strong termination sites, or regions, for these genes. We cannot rule out other more complicated explanations for the abrupt loss of polymerases and transcripts (Moss *et al.*, 1985; Labhart and Reeder, 1986), but consider them unlikely as discussed below. Certain other non-chorion transcription units display alternate transcript patterns in the 3' region, as would be expected in the case of multiple inefficient termination sites. For example, Figure 2 shows a gene in which the 5' portion is densely loaded with polymerase molecules and associated transcripts, while the 3' portion of the gene is only sparsely loaded. If we are observing a gene with multiple termination sites, it is apparent that the remaining RNA polymerase molecules are stable to our spreading conditions after traversing a putative termination region.

For the purposes of this study, we addressed the following questions: how uniform are the *s36* and *s38* transcription unit lengths; is there any decrease in polymerase density at the 3' ends of the genes; and where are the termination sites with respect to the known polyadenylation sites? We measured 20 gene pairs that were amenable to analysis. Because the polymerase density is very high on both chorion genes, transcription unit length (in μm) was determined by measuring the template from first polymerase to last polymerase. The absolute measurements for these gene pairs are mapped in Figure 1c in order of increasing *s36* length. The mean lengths ($\pm$ SD) are $0.367 \pm 0.033 \mu\text{m}$ for *s36*, $0.340 \pm 0.038 \mu\text{m}$ for the spacer and $0.590 \pm 0.064 \mu\text{m}$ for *s38*. A rigorous approach to the question of the transcription unit length uniformity is complicated by the fact that when

Fig. 1. Analysis of *s36* and *s38* chorion gene lengths and positions of RNA polymerase II molecules. (a) Map of the *s36* and *s38* chorion gene locus giving gene and spacer lengths in nucleotides, i.e. from +1 to poly(A) addition sites (B.Wakimoto, J.Levine and A.Spradling, personal communication). (b) Electron micrograph showing a transcriptionally active *s36* and *s38* chorion gene pair. The photograph is cropped to eliminate background chromatin. Bar = 0.2 μ m. (c) Gene and spacer lengths of 20 *s36* and *s38* gene pairs. The genes are plotted according to increasing *s36* gene length. Lengths in μ m are presented for each unit. (d) Histogram showing total distribution of RNA polymerase II molecules across the *s36* and *s38* gene pairs displayed in (e). The 20 *s36* genes are transcribed by a total of 241 RNA polymerase II molecules distributed, in the 5' to 3' direction, into quarters containing 59, 53, 64 and 65 polymerase molecules respectively. The 20 *s38* genes are bound by 450 RNA polymerase II molecules, with each quarter containing 112, 110, 123 and 105 molecules respectively from 5' to 3'. (e) Lengths of *s36* and *s38* chorion genes and spacers presented in (c), normalized and showing the location of RNA polymerase II molecules. The distance from the 5' end of *s36* to the 5' end of *s38* is constant for each gene pair (2516 bp). On this basis, each *s36* gene and spacer length in (c) is normalized to 1 and replotted. A ratio of actual to normalized length of each *s36* gene is determined and used to obtain a corresponding *s38* length, assuming there is equal compaction in the transcribed region of the two linked genes.

Fig. 2. An unidentified, transcriptionally active gene from a chromatin spread of stage 12 *Drosophila* follicle cells. The gene is characterized by a high transcript density in the 5' half of the transcription unit (about 23 transcripts - large arrow) and a low transcript density (three transcripts - small arrows) in the 3' portion. This is the transcriptional pattern one would expect if termination of the gene occurred at multiple, weak sites extending over 1 kb or more, as has been found for mouse and rabbit β -globin genes and the mouse α -amylase gene (Citron *et al.*, 1984; Hagenbuehle *et al.*, 1984; Rohrbaugh *et al.*, 1985). Bar = 0.2 μ m.

chromatin is dispersed by our method, it can be stretched or compacted to varying degrees as it is deposited and fixed on the grid. The above measurements, however, are consistent with a single termination site based on comparison to ribosomal DNA gene lengths measured under similar conditions. The rDNA gene is useful as a reference multi-copy gene of high polymerase density and known length (i.e. from the transcription initiation site to an RNA cleavage site which is coincident with the 3' end of the mature 28S ribosomal RNA) (Trendelenburg, 1982; Labhart and Reeder, 1986). Our standard deviations in gene length, considered as a fraction of the mean gene lengths, are $\pm 9\%$ for *s36* ($n = 20$) and $\pm 10.8\%$ for *s38* ($n = 20$). For *Xenopus* rDNA genes, the standard deviations derived for two samplings represented 12% ($n = 24$) and 14.5% ($n = 26$) of the mean gene length (Bakken *et al.*, 1982). Thus, our measurements are consistent with a unique termination site for the two genes. It is of interest that the ratio of *s36*:*s38* primary transcription unit lengths is 0.62 (0.367 μ m/0.590 μ m), whereas the ratio of gene lengths determined from DNA sequencing (i.e. from the transcription initiation sites to the poly(A) addition sites) is 0.73 (1100 bp/1516 bp). There are two important implications of this finding. First, the *s38* termination site is further downstream from its poly(A) site than is the *s36* site, and second, we are not simply visualizing and mapping cleavage at the polyadenylation sites.

Figure 1e displays normalized *s36* and spacer lengths for the same gene pairs shown in Figure 1c. This length, from the start site of *s36* to the start site of *s38*, is constant (2516 bp) and can be fairly precisely measured in our micrographs. The transcribed DNA occupies 52% of this region on average with a standard deviation of 7% of its length. If *s36* terminates at multiple sites, one would expect to see the distance occupied by the spacer shorten as the *s36* transcription unit, as measured in μ m, lengthens. However, Figure 1e shows that as *s36* gets longer (from top to bottom in the figure), there is only a slight tendency for the distance occupied by the spacer to shorten, i.e. there is only a weak positive correlation between *s36* gene length (μ m) and the ratio of *s36* length to spacer length ($r = 0.58$). The primary observation from Figure 1c and e is that the transcribed regions are fairly uniform in length and that a transcript-free spacer occupies 40–50% of the *s36* spacer region, visually reinforcing the existence of a discrete termination site or region for *s36*.

Fig. 3. The total number of RNA polymerase II molecules per transcription unit is plotted according to actual gene length in μ m. (a) The *s36* gene lengths and associated RNA polymerase II molecules presented in Figure 1c and e are plotted in relation to each other. Correlation coefficient = 0.20. (b) is the corresponding analysis for the *s38* genes in Figure 1c and e. Correlation coefficient = 0.48.

Because of the high polymerase density on these genes, one would expect to see more polymerases transcribing the longer genes if they indeed represent longer transcription units rather than genes that have been stretched a bit more in grid preparation. However, as shown in Figure 3, the total number of polymerases per gene does not increase with increasing gene length.

A decrease in polymerase density at the 3' end of the genes would be expected if multiple termination sites spread out over some distance were used. As shown in the histograms in Figure 1d, polymerase density is fairly constant across the transcription unit. Actual polymerase positions are shown on the gene maps in Figure 1e. If there are multiple termination sites, they occur within a few hundred basepairs, as discussed below, and at least one of them is quite efficient.

Based on the following criteria, we conclude that there is a discrete and efficient termination region, or possibly a single termination site, for the *s36* and *s38* chorion genes: (i) the uniformity in gene lengths in both an absolute and normalized sense; (ii) the fairly constant polymerase number on individual copies of the genes; and (iii) the uniformity in polymerase density across the transcription units. Given standard deviations of $\pm 10\%$ of gene length and a gene length of about 1.2 kb for *s36* (see below), this method allows localization of the termination region to about 240 bp, which is comparable to the best termination mappings to date using the nuclear run-on method (Rohrbaugh *et al.*, 1985; LeMeur *et al.*, 1984; Sheffery *et al.*, 1984).

Where are the observed termination sites with respect to the known polyadenylation sites, which fall at position 1100 for *s36* and position 1516 for *s38* (B. Wakimoto *et al.*, personal communication)? To answer this question, the measured lengths (in μ m) must be converted to lengths in base pairs. This conversion is complicated by the above-mentioned variation in compaction of chromatin when deposited on the grids. We will give two extremes in conversion factors representing lower and upper limits for gene lengths. Below, we argue that the actual length is probably close to the middle of the range.

It has been reasonably argued that chromatin compaction in transcribed regions is inversely related to polymerase density (McKnight *et al.*, 1978). This argument is based in part on the presence of nucleosomes between polymerases on sparsely transcribed genes, and their absence on heavily transcribed genes like the chorion genes. Nucleosomal chromatin is compacted ~1.9-fold over B form DNA in these spreads, whereas heavily transcribed ribosomal genes are compacted only 1.1- to 1.2-fold relative to B form (Laird *et al.*, 1976). Thus, our lowest gene length estimates are determined by assuming that the transcribed chorion DNA is stretched to B form in our spreads (2.94 kb/ μ m). This yields average transcription unit lengths of 1079 for *s36* ($n = 20$) and 1735 for *s38* ($n = 20$). This is actually slightly shorter than the expected minimum transcription unit length for *s36*, which extends 1100 bp to the poly(A) site. In addition, other studies have shown a requirement for transcription of an additional 20–40 nucleotides downstream of the poly(A) site for correct 3' cleavage/polyadenylation (Gil and Proudfoot, 1984; Hart *et al.*, 1985). Thus, this estimate of gene length (assuming B form conformation) is indeed a lower limit.

Upper limits for gene lengths were obtained by converting the length in μ m from the start of *s36* to the start of *s38* (i.e. *s36* gene plus spacer) to 2516 bp, its known sequenced length. This procedure assumes equal chromatin compaction in the gene and spacer regions and yields an average length of 1306 bp (± 117 bp) for *s36*, with an average compaction ratio of 3.6 kb/ μ m (range 3.1–4.1). Using the appropriate ratio to convert the adjacent *s38* measurement to base pairs yields an average of 2100 bp (± 228) for *s38*. The assumption of equal chromatin compaction in the gene and spacer regions, however, is very unlikely since nucleosomes are typically present on the inter-gene spacer DNA, suggesting a compaction greater than the non-nucleosomal gene.

We conclude that the actual transcription unit lengths fall between 1100 and 1306 bp for *s36* and between 1735 and 2100 bp for *s38*. The actual lengths are probably near the middle of the range, again based on comparison to the rDNA gene. That is, the *Drosophila* ribosomal gene has a compaction of 3.3 kb/ μ m, which is about halfway between 2.94 and 3.6 kb/ μ m, our lower and upper limits. Polymerase density is very high and similar on the chorion genes (45 pol/ μ m) and the *Drosophila* rDNA gene (50 pol/ μ m) (Osheim *et al.*, 1985; McKnight and Miller, 1976). Using the 3.3 kb/ μ m conversion factor, average lengths of 1211 bp for *s36* and 1947 bp for *s38* are obtained, which we consider our best estimates. In any case, the possible ranges of gene length based on the limits described are not large: 210 bp for *s36* and 365 bp for *s38*. The major conclusion is that the termination region is very near the poly(A) site for *s36* (0–210 bases downstream), but somewhat further from the poly(A) site for *s38* (220–585 bases downstream). This difference between the two genes was suggested by measurements of transcription unit lengths, as discussed previously. The chromatin compaction in the spacer between the genes can be deduced from these calculations to be about 1.3-fold over B form DNA or 3.8 kb/ μ m. This is less than the 1.9-fold compaction previously estimated for nucleosomal chromatin (Laird *et al.*, 1976) but is similar to the 1.4-fold compaction seen in the spacer of *Xenopus* ribosomal genes (Pruitt and Grainer, 1981). The chorion gene spacer contains the *s38* promoter and may be involved in conferring tissue specificity and in controlling the amplification event (Wakimoto *et al.*, 1983; Parks *et al.*, 1982).

Figure 4 summarizes the termination patterns of the non-viral and non-yeast *Pol*II genes examined to date; all except the sea urchin *H2A* histone gene are polyadenylated. The genes vary considerably in the extent of RNA transcribed at normal levels 3' to the gene and in the size of the region over which termination

Fig. 4. Comparison of transcription termination patterns exhibited by genes transcribed by RNA polymerase II. Genes from transcription start site (left) to final poly(A) site (right) are indicated by black boxes. The straight, dashed lines extending from the end of the genes represent regions that are transcribed at the same level as within the gene regions. The tapering, dashed lines indicate the region over which transcription declines and ceases. Nucleotide lengths of the transcribed regions beyond the 3' end of the genes are given in bp above their respective regions. Non-transcribed spacer lengths are also shown. Genes utilizing two or more poly(A) sites have the major poly(A) site(s) demarcated by a large arrow. Minor poly(A) sites are indicated by small arrows. The sea urchin *H2A* histone gene is not polyadenylated. Termination patterns are displayed for 12 genes: the *Drosophila s36* and *s38* chorion, mouse $\alpha 1$ globin (Sheffery *et al.*, 1984), chicken α_D and α_A globin (Weintraub *et al.*, 1981), mouse β^{maj} globin (Citron *et al.*, 1984; Falck-Pedersen *et al.*, 1985), rabbit $\beta 1$ globin (Rohrbaugh *et al.*, 1985), mouse α -amylase *Amy 2^a* (Hagenbuchle *et al.*, 1984), chicken ovalbumin (LeMeur *et al.*, 1984), mouse dihydrofolate reductase (Frayne *et al.*, 1984), rat calcitonin (Amara *et al.*, 1984) and sea urchin *H2A* histone (Birchmeier *et al.*, 1984).

occurs. The actual distance between poly(A) sites and termination sites, and the efficiency of the termination site(s), seem unimportant for correct 3' terminal processing. One restraint on the position and efficiency of termination sites may be the occurrence of a nearby downstream gene since three of the genes that terminate near their 3' mRNA termini are closely linked to downstream genes (*s36* chorion, chick α_D globin and *H2A* histone genes).

The chorion gene termination patterns resemble those in several other genes in the rapid fall-off of transcription over a short stretch of DNA (Figure 4). This major termination region in the chicken ovalbumin gene is positioned about 1000 nucleotides downstream of its major poly(A) site. More than 90% of the transcripts terminate within the 170-bp stretch. The remainder of the transcripts continue for another 600 bases before terminating over a short region just beyond a minor polyadenylation site (LeMeur *et al.*, 1984). If a similar situation existed for the *s36* chorion gene, i.e. if transcription termination were only 90% efficient at the site we have mapped and the other 10% of the transcripts continued on throughout the spacer, we would expect to see on average 1–2 transcripts and/or polymerases in the inter-gene region. In fact, we see no transcripts in the 20 inter-gene spacers we have mapped. There are, however, a total of nine particles in these 20 spacers which are either larger than nucleosomes, or the correct size for nucleosomes but with the characteristic dark staining property of polymerase molecules. These particles are not found at the 3' ends of the *s36* genes, but rather occur across the entire spacer. We can calculate then that termination is at least 97%, and perhaps 100%, efficient at the site we have mapped at the end of the *s36* gene, depending on how many of the particles are actually RNA polymerase II molecules. If these are polymerases, they are either not transcribing or their transcripts are extremely unstable.

Some recent studies have called into question the validity of using EM spreading to map termination sites. Specifically, studies of nuclear run-on transcription in *Xenopus* (Labhart and Reeder, 1986) and *Drosophila* (Tautz and Dover, 1986) ribosomal gene regions have concluded that all or most of the 'non-transcribed' inter-gene spacer is actually transcribed, albeit into very unstable RNA which cannot be detected in the steady-state RNA population. EM chromatin spreads of rDNA genes from both *Xenopus* (Miller and Beatty, 1969; Trendelenburg, 1982) and *Drosophila* (McKnight and Miller, 1976) show occasional short transcripts or polymerases in these spacers, but, for the most part they appear nucleosomal (Martin *et al.*, 1980), free of polymerases and transcripts, and certainly not transcribed to levels equivalent to the gene regions as shown for *Drosophila* by Tautz and Dover (1986) from nuclear run-on experiments. If these latter experiments faithfully reflect the *in vivo* situation, then it must be argued that the *PoII* interaction with the DNA template is somehow different in the intergene versus gene regions such that it is preferentially destabilized in the inter-gene region by the chromatin spreading method. There are examples of abnormal rDNA spacer transcription in *Xenopus* oocytes, however, resulting from either initiation in the spacer (Trendelenburg, 1981) or transcription through the spacer (Trendelenburg *et al.*, 1978), in which the spacer polymerases and transcripts are stable to EM spreading conditions. As an alternative explanation for the apparent spacer transcription, we consider it quite possible that nuclei which have been isolated and RNase treated for run-on experiments (Tautz and Dover, 1986) have lost essential termination factors. We cannot at this time resolve the obvious discrepancies between the EM and biochemical approaches in

terms of *PoII* transcription. Whether or not such a discrepancy will exist for *PoIII* genes is not yet known. It is worth considering, however, that for ribosomal genes, electron microscopy reveals the formation of the functional 3' end of the rRNA precursor molecule (Trendelenburg, 1982). Although we cannot rigorously rule out that the sites we have mapped correspond to cleavage rather than termination sites, we feel strongly that they represent the functional 3' ends of the chorion primary transcripts and that they do not correspond to the poly(A) cleavage sites.

The molecular characterization of *PoIII* termination sites is at an early stage. The sea urchin histone *H2A* site has been localized to a 130-bp AT-rich region by deletion mapping (Birchmeier *et al.*, 1984), and a 1395-bp fragment downstream of the mouse β globin gene causes termination when inserted into a viral gene (Falck-Pedersen *et al.*, 1985). Understanding the events involved in producing a 3' terminus are basic to the study of gene regulation, since the generation of diverse 3' ends through termination and/or processing can influence the splicing pattern of particular molecules, leading to the generation of alternate proteins (Nevins, 1983; Mather *et al.*, 1984; Rosenfield *et al.*, 1984).

This EM method of termination mapping is applicable to any highly transcribed *PoIII* gene that can be identified in a chromatin spread. Localization of genes on introduced plasmid vectors (Bakken *et al.*, 1982; Osheim and Beyer, 1985) will greatly aid in identification and will permit mutational analysis of the termination region.

Materials and methods

D. melanogaster follicle cell chromatin was prepared for EM as previously described (Osheim and Miller, 1983). Ovaries from *D. melanogaster* Oregon-R females were dissected out over ice in pH 9.0 water. An egg chamber of the appropriate stage was removed from the ovariole and the end containing the nurse cells was squeezed off. The remainder of the egg chamber was transferred to 100 μ l of 0.05% Joy, 0.05% Na-desoxycholate (pH 8.2), where the follicle cells were disrupted. After 20–40 min of chromatin dispersal, 25 μ l of 0.1 M sucrose, 10% formalin (pH 8.5), were added, and the solution was spun through a sucrose cushion onto a carbon-coated EM grid. The grids were stained with phosphotungstic acid and uranyl acetate and then rotary shadow-cast with platinum.

Acknowledgements

We thank Drs Allan Spradling, Barbara Wakimoto and Joe Levine for communicating sequence information prior to publication. This work was funded by grants from the National Science Foundation (PCM-83-09131) and the American Cancer Society (NP-493) to A.L.B. and by a grant from the National Institutes of Health (GM 21020) to O.L.M.

References

- Amara, S.G., Evans, R.M. and Rosenfield, M.G. (1984) *Mol. Cell. Biol.*, **4**, 2151–2160.
- Bakken, A., Morgan, G., Sollner-Webb, B., Roan, J., Busby, S. and Reeder, R.H. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 56–60.
- Beyer, A.L., Bouton, A.H. and Miller, O.L., Jr (1981) *Cell*, **26**, 155–165.
- Birchmeier, C., Schumperli, D., Sconzo, G. and Birnstiel, M.L. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 1057–1061.
- Birnstiel, M.L., Busslinger, M. and Strub, K. (1985) *Cell*, **41**, 349–359.
- Citron, B., Falck-Pedersen, E., Salditt-Georgieff, and Darnell, J.E., Jr (1984) *Nucleic Acids Res.*, **12**, 8723–8731.
- Falck-Pedersen, E., Logan, J., Shenk, T. and Darnell, J.E., Jr. (1985) *Cell*, **40**, 897–905.
- Ford, J. and Hsu, M.-T. (1978) *J. Virol.*, **28**, 795–801.
- Frayne, E.G., Leys, E.J., Crouse, G.F., Hook, A.G. and Kellems, R.E. (1984) *Mol. Cell. Biol.*, **4**, 2921–2924.
- Gil, A. and Proudfoot, N.J. (1984) *Nature*, **312**, 473–474.
- Hagenbuchle, O., Wellauer, P.K., Cribbs, D.L. and Schibler, U. (1984) *Cell*, **38**, 737–744.
- Hart, R.P., McDevitt, M.A. and Nevins, J.R. (1985) *Cell*, **43**, 677–683.
- Labhart, P. and Reeder, R.H. (1986) *Cell*, **45**, 431–443.

- Laird,C.D. and Chooi,W.Y. (1976) *Chromosoma*, **58**, 193–218.
- Laird,C.D., Wilkinson,L.E., Foe,V.E. and Chooi,W.Y. (1976) *Chromosoma*, **58**, 169–192.
- LeMeur,M.A., Galliot,B. and Gerlinger,P. (1984) *EMBO J.*, **3**, 2779–2786.
- Martin,K., Osheim,Y.N., Beyer,A.L. and Miller,O.L.,Jr (1980) In McKinnel,R.G., Di Berardino,M.A., Blumenfeld,M. and Bergad,R.D. (eds), *Differentiation and Neoplasia in Results and Problems in Cell Differentiation*. Springer-Verlag, Berlin, Vol. 11, pp. 37–44.
- Mather,E.L., Nelson,K.J., Haimovich,J. and Perry,R.P. (1984) *Cell*, **36**, 329–338.
- McKnight,S.L. and Miller,O.L.,Jr (1976) *Cell*, **8**, 305–319.
- McKnight,S.L., Bustin,M. and Miller,O.L.,Jr (1978) *Cold Spring Harbor Symp. Quant. Biol.*, **42**, 741–754.
- Miller,O.L.,Jr and Beatty,B.R. (1969) *Genetics Suppl.*, **61**, 133–143.
- Moss,T., Mitchelson,K. and deWinter,R. (1985) In Maclean,N. (ed.), *Oxford Surveys of Eukaryotic Genes*. Oxford University Press, Oxford, Vol. 2, pp. 207–250.
- Nevins,J.R. (1983) *Annu. Rev. Biochem.*, **52**, 441–466.
- Nevins,J.R. and Darnell,J.E.,Jr (1978) *Cell*, **15**, 1477–1493.
- Orr,W., Komitopoulou,K. and Kafatos,F.C. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 3773–3777.
- Osheim,Y.N. and Miller,O.L.,Jr (1983) *Cell*, **33**, 543–553.
- Osheim,Y.N. and Beyer,A.L. (1985) in Smuckler,E.A. and Clawson,G.A. (eds), *Nuclear Envelope Structure and RNA Maturation in UCLA Symposia on Molecular and Cellular Biology*. Alan R.Liss, New York, pp. 277–295.
- Osheim,Y.N., Miller,O.L.,Jr and Beyer,A.L. (1985) *Cell*, **43**, 143–151.
- Parks,S., Kalfayan,L. and Spradling,A. (1982) *Carnegie Inst. Washington Year Book*, **81**, 190–194.
- Petri,W.H., Wyman,A.R. and Kafatos,F.C. (1976) *Dev. Biol.*, **49**, 185–189.
- Pruitt,S.C. and Grainger,R.M. (1981) *Cell*, **23**, 711–720.
- Rohrbaugh,M.L., Johnson,J.E.,III, James,M.D. and Hardison,R.C. (1985) *Mol. Cell. Biol.*, **5**, 147–160.
- Rosenfield,M.G., Amara,S.G. and Evans,R.M. (1984) *Science*, **225**, 1315–1320.
- Sheffery,M., Marks,P.A. and Rifkind,R.A. (1984) *J. Mol. Biol.*, **172**, 417–436.
- Spradling,A.C. (1981) *Cell*, **27**, 193–201.
- Spradling,A.C. and Mahowald,A.P. (1979) *Cell*, **16**, 589–598.
- Spradling,A.C. and Mahowald,A.P. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 1096–1100.
- Tautz,D. and Dover,G.A. (1986) *EMBO J.*, **5**, 1267–1273.
- Trendelenburg,M.F. (1981) *Biol. Cell.*, **42**, 1–12.
- Trendelenburg,M.F. (1982) *Chromosoma*, **86**, 703–715.
- Trendelenburg,M.F., Zentgraf,H., Franke,W.W. and Gurdon,J.B. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 3791–3795.
- Wakimoto,B., Kalfayan,L. and Spradling,A. (1983) *Carnegie Inst. Washington Year Book*, **82**, 198–202.
- Waring,G.L. and Mahowald,A.P. (1979) *Cell*, **16**, 599–607.
- Weintraub,H., Larsen,A. and Groudine,M. (1981) *Cell*, **24**, 333–344.

Received on 17 September 1986