

the low optical luminosity — a 'warmer', which is a cluster of highly evolved, hot, massive stars. R. Terlevich and J. Melnick suggested (*Mon. Not. R. astr. Soc.* **213**, 841–856; 1985) that such an object, in which stars could have effective temperatures as high as 100,000 K, could easily be mistaken for a Seyfert nucleus, having the same characteristics of ionizing radiation. The positive identification of one warmer would greatly support the hypothesis that many other galaxies have mistakenly been identified as Seyferts. The biggest problems in applying the warmer model to the nucleus of NGC4395 are accounting for the high velocities seen in the permitted lines, which imply extremely energetic outflow, for which no mechan-

ism seems to be available, and the lack of spectral features seen in Wolf-Rayet stars, thought to be akin to the stars in a warmer.

Finally, if the nucleus studied by Filippenko and Sargent is in the family of accretion-driven Seyfert galaxies, it implies that even low-mass galaxies can be hosts to active nuclei, and that such faint nuclei may be common and are unknown because they are apparent in only the nearest dwarf galaxies. It is hoped that further studies of the nuclei of similar galaxies will clarify the relationship between galaxy type and activity. □

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DROSOPHILA DEVELOPMENT

Making stripes inelegantly

Michael Akam

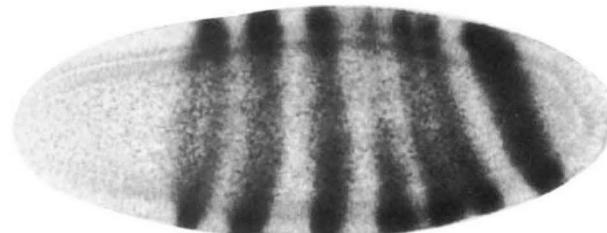
A STRIKING feature of *Drosophila* embryogenesis is the early appearance along the antero-posterior (A-P) axis of a precisely repeating pattern, the stripes of expression of the pair-rule genes^{1,2} (see figure). This pattern depends upon the earlier expression of the segment gap genes, but these do not provide periodic cues; different gap genes are expressed in zones of varying width along the axis of the embryo. Periodicity might be generated in one of two ways. An elegant mechanism, favoured by model builders^{3,4}, would use an intrinsically periodic pattern-

interaction with a gradient of the protein expressed from the *bicoid* gene; successively more posterior regions of the embryo express *Krüppel* (*Kr*), then *knirps* (*kni*)^{5,6}. When the distribution of transcripts from these genes is analysed, the region expressing each appears to be sharply bounded, defining abutting¹⁰ or slightly overlapping¹¹ zones of expression. But the protein products of the gap genes are not so precisely localized. Antibodies reveal that the hunchback and Krüppel proteins accumulate in broader domains with no well-defined boundaries (see

Fig. 4 of Stanjovec *et al.* on page 334 of this issue⁸). The apparent limits of these domains depend considerably on the sensitivity of the antibody staining techniques used¹¹. Even so, it is clear that they overlap substantially.

Within these regions of overlap, the relative concentrations of two gap proteins change continuously over a distance of at least 8–10 nuclei (about 2–3 segment primordia). It seems very likely that these graded changes in concentration and/or ratios of the gap gene products are providing cues for subsequent pattern generation.

Genetic studies indicate that all of the pair-rule genes are dependent on the gap proteins for normal patterning, but that only three 'primary' pair-rule genes, *hairy*, *runt* and *even-skipped* (*eve*), are absolutely required to generate the periodic pattern². Two of these primary pair-rule genes have now been shown to contain discrete promoter elements that mediate the generation of different parts of the stripe pattern. The first hint of this



Stripes on a *Drosophila* embryo — in this case somewhat abnormal *ftz* stripes (from ref. 22).

generating system, comprising the pair-rule genes and their products. This would only need to be triggered by local stimuli from the gap genes. Alternatively, unique instructions could be generated by the gap-gene proteins to define the position of each pair-rule stripe. Analysis of the interaction between gap and pair-rule genes seems to favour the less elegant 'specific instruction' process. Discrete elements within promoters for the pair-rule genes contain specific targets for the binding of one or more gap-gene proteins. These elements direct the expression of single stripes along the A-P axis⁵.

The expression of three gap genes spans the greater part of the segment pattern. The *hunchback* (*hb*) gene is activated in the anterior half of the embryo by direct

came from the study of mutations at the *hairy* locus⁵. Whereas most *hairy* mutants display segment fusions throughout the whole embryo, a subset of mutants causes segment fusion only in particular regions of the embryo. These mutants turned out to have deletions of different parts of the 5'-regulatory region of *hairy*. This implied the presence of region-specific promoter elements, in marked contrast to the organization of the promoter for the 'secondary' pair-rule gene *fushi-tarazu* (*ftz*)¹². At *ftz*, promoter deletions generate either the complete set of stripes, or no stripes. This different organization supports the view that *hairy* responds directly to signals from the gap genes, whereas *ftz* responds only to periodic signals, generated by the pair-rule genes themselves.

A series of papers dissecting the regulation of the *eve* locus have amplified and confirmed this model^{6,8,13}. The *eve* promoter proves to be particularly complex, in that it has both gap-response elements that mediate its initial activation, and a functionally independent element that responds to the accumulating products of the pair-rule genes. The pair-rule element becomes active somewhat later than the gap-response elements, and directs a distinct pattern of slightly narrower stripes.

So far, the gap-response elements have only been defined by deletion: regions of 1–2 kilobases of DNA within the 5'-regulatory region of *eve* specifically activate either stripe 3 or stripes 2 and 7 of the *eve* expression pattern. When truncated promoters containing the gap-response elements, but not the pair-rule response element, are re-inserted into embryos, they mimic very precisely the early expression of the endogenous *eve* gene in stripes 2, 3 and 7 (ref. 6). This activity remains dependent on the gap-gene products, but is unaffected by mutations in the pair-rule genes, implying that input from the gap genes alone can specify the location and width of the initial *eve* stripes.

It has been postulated for some time that the three gap genes, *hb*, *Kr* and *kni*, may be acting directly as transcription factors. Each encodes a putative zinc-finger protein^{14–16} consistent with that notion. Strong evidence for this view is provided by three papers in this issue^{8,17,18}. These demonstrate that the hunchback and Krüppel proteins bind to specific DNA targets. One of these studies examines the binding of these gap proteins to the *eve* promoter. Using footprinting techniques, Stanjovec *et al.*⁸ find multiple binding sites for both proteins. Most of these are clustered within the elements that direct specific stripe formation.

The two other papers examine binding of the gap proteins to different potential target sequences. Treisman and Desplan¹⁷ identify hunchback and Krüppel binding

sites upstream of the *hb* promoter; Pankratz *et al.*¹⁸ define Krüppel binding sites upstream of the *kni* promoter. In each case, a very similar DNA sequence characterizes the Krüppel binding site, although *Kr* has been implicated as a negative regulator of *hb*, but a positive regulator of *kni*. The proposed hunchback binding sequences are more divergent. (It should be noted that there is no evidence that the hunchback protein binds to its own promoter, so the sequences defined by Treisman and Desplan¹⁷ need not be functional.)

If Krüppel is acting directly by binding to the promoters of *kni*, and of the pair-rule genes, as these results suggest, then it must be functional at concentrations where the protein is barely detectable by present techniques. Pankratz *et al.* point out that an effect of Krüppel is observed throughout the zone of *kni* transcription, even though part of this lies beyond the detectable limits of Krüppel protein. *Kr* mutants alter the pattern of *eve* transcription in a region spanning *eve* stripes 2–6 (ref. 13), again suggesting that Krüppel is effective at, or beyond, the apparent limits of its distribution. This evidence for the action of the gap proteins at low concentrations throughout relatively large numbers of segment primordia, raises the intriguing possibility that the gap genes are effectively generating secondary gradients within the egg, extending from the boundaries of their zones of transcription.

The gap genes have been implicated in the primary regulation of the homoeotic genes, as well as in the segmentation hierarchy^{1,19,20}. If the gap proteins are functional over a wide concentration range, they could provide positional information for the definition of precise segment identities, as well as for the positioning of the pair-rule stripes. Such

information must be available within the blastoderm, but the discrete domains of gap-gene expression do not appear to provide sufficient resolution for this purpose. Local gradients of gap protein concentration could, however, provide the needed resolution.

If each pair-rule stripe is generated by the interpretation of a unique instruction, then the apparent simplicity of the repeating segment pattern is deceptive. A 'specific instruction' mechanism could generate an arbitrarily complex set of uneven stripes. The simplicity of the natural pattern must then be attributed to a process of selection needed to produce a

set of evenly sized segment primordia.

Where does this leave spontaneous pattern-generating mechanisms? One very likely role for these processes is to amplify the initial discontinuities in pair-rule gene expression, and to sharpen the boundaries between stripes²¹. In this respect, the feedback circuits of the pair-rule genes act as bistable, or perhaps multi-stable, switches, forcing cells into one of a few preferred states. But it seems that they do not define periodicity. □

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ATMOSPHERIC OZONE

Ultraviolet levels down not up

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Most people are familiar with the danger of ozone depletion in the upper atmosphere, and its probable consequences. Fewer are aware that the concentration of ozone in the lower atmosphere may be increasing. According to C. Brühl and P. J. Crutzen, in a recent issue of *Geophysical Research Letters* (16, 703–706; 1989), this increase could counteract the effect of the stratospheric depletion on the amount of ultraviolet radiation reaching the Earth's surface, especially in the Northern Hemisphere. This could mean that the increased incidence of skin cancer that many fear will follow stratospheric depletion, may not materialize. Instead, the effect in the changing concentrations would be seen most in atmospheric temperatures, with a warming of the troposphere and a cooling of the stratosphere.

Interest in the behaviour of tropospheric ozone is growing strongly, because it is an efficient greenhouse gas, it probably causes damage to vegetation at elevated concentrations and it has a large influence on the chemical reactivity of the troposphere through its ability to generate hydroxyl radicals on photolysis. It is now becoming accepted that the quantity of ozone is increasing in much of the troposphere. According to reports at a recent meeting*, ozone levels are increasing over both the continents and the oceans in the Northern Hemisphere. Also, it seems, the various chemical precursors of ozone — hydrocarbons and nitrogen oxides — are now spread throughout the troposphere. Observations of substantial concentrations of hydrogen peroxide, whose precursors react with nitrogen oxides to give ozone, underline this view of tropospheric ozone.

Brühl and Crutzen's new work concerns the different effect that tropospheric and stratospheric ozone have on the

biologically active ultraviolet radiation (UVB) from the Sun. They argue that the path of light is less direct through the troposphere than it is through the stratosphere: this is because molecules, cloud droplets and aerosols, which are present in greater density in the lower atmosphere, scatter the light rays. The path length through the troposphere is thus relatively increased and each ozone molecule is more likely to intercept and absorb light.

The authors calculate the effect of this mechanism using data from the Hohenpeissenberg Observatory in Bavaria on the ozone concentration from 0–10 km (the troposphere) and above 10 km (the stratosphere). They find that between 1968 and 1982, the UVB reaching the Earth's surface actually decreased by 0.9 per cent at noon and by 0.5 per cent throughout the day. If a weighting factor is applied to the proportion of the UVB that interacts with DNA, the decreases calculated for noon and the whole day at the summer solstice become 1.7 and 0.9 per cent, respectively. This occurs in spite of an overall decrease in the ozone column density of 1.5 per cent, attributable to stratospheric depletion, over the same period. The contribution made by ozone in the troposphere is much more effective at lower solar zenith angles. At high solar zenith angles, which is the case for the winter months at high latitudes, the scattering of UVB mostly occurs in the traverse through the extended path length in the stratosphere.

Brühl and Crutzen emphasize the role played by increased absorption of ultraviolet by ozone throughout large parts of the remote troposphere. Its effect will be much more pronounced in the Northern Hemisphere where ozone concentrations have increased substantially over the past 50 years (S.A. Penkett in *The Changing Atmosphere* (eds F.S. Rowland & I.S.A.

1. Akam, M.E. *Development* **101**, 1–22 (1987).
2. Ingham, P.W. *Nature* **335**, 25–34 (1988).
3. Meinhardt, H. *J. Cell Sci. suppl.* **4**, 357–381 (1986).
4. Lacalli, T.C., Wilkinson, D.A. & Harrison, L.G. *Development* **103**, 105–113 (1988).
5. Howard, K., Ingham, P. & Rushlow, C. *Genes Dev.* **2**, 1037–1046 (1988).
6. Goto, T., Macdonald, P. & Mariotti, T. *Cell* **57**, 413–422 (1989).
7. Harding, K., Hoey, T., Warrior, R. & Levine, M. *EMBO J.* **8**, 1205–1212 (1989).
8. Stanjovec, D., Hoey, T. & Levine, M. *Nature* **341**, 331–335 (1989).
9. Howard, K. *Nature* **338**, 618–619 (1989).
10. Jackie, H. *et al.* *Nature* **324**, 668–670 (1986).
11. Gaul, U. & Jäckle, H. *Development* (in the press).
12. Hiromi, Y. & Gehring, W. *Cell* **50**, 963–974 (1987).
13. Frasch, M. & Levine, M. *Genes Dev.* **1**, 981–995 (1987).
14. Knipple, D.C., Seifert, E., Rosenberg, R.B., Preiss, A. & Jäckle, H. *Nature* **317**, 40–44 (1985).
15. Tautz, D. *et al.* *Nature* **327**, 383–389 (1987).
16. Nauber, U. *et al.* *Nature* **336**, 489–492 (1988).
17. Treisman, J. & Desplan, C. *Nature* **341**, 335–337 (1989).
18. Pankratz, M.J., Hoch, M., Seifert, E. & Jäckle, H. *Nature* **341**, 337–340 (1989).
19. Harding, K. & Levine, M. *EMBO J.* **7**, 205–214 (1988).
20. Irish, V.L., Martinez-Arias, A. & Akam, M. *EMBO J.* **8**, 1527–1537 (1989).
21. Edgar, B.A., O'Dell, G.M. & Schübler, G. *Dev. Genet.* **10**, 124–142 (1989).
22. Hülsmann, M. *et al.* *Nature* **338**, 629–632 (1989).

* *Tropospheric Ozone*, Norwich, 3–7 July 1989.