Decoding positional information: regulation of the pair-rule gene *hairy*

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Summary

In the series of local gene activations that occur during early *Drosophila* development, the striped expression patterns of the pair-rule genes provide the first indication of segmental periodicity. The experiments that we report here address the question of how these patterns arise, by studying the regulation of one of these genes, *hairy*. We show that each of the seven stripes of *hairy* expression is controlled by a distinct subset of cis-acting regulatory elements, some mediating transcriptional activation and others transcriptional repression. In general, elements necessary and sufficient for triggering a particular stripe response are clustered on the DNA and appear to overlap or be interspersed with elements involved in at least one other stripe response. Our results extend previous findings suggesting that periodic *hairy* expression arises by a decoding process in which each stripe is triggered by particular combinations or concentrations of regulatory factors. These regulatory factors are likely to include the products of the gap class of segmentation genes that are required for activating or positioning particular subsets of *hairy* stripes and are expressed with overlapping distributions during early embryogenesis.

Key words: *Drosophila*, segmentation, transcriptional regulation.

Introduction

During *Drosophila* oogenesis, the primary determinants of anteroposterior body pattern are deposited at the poles of the maturing egg (reviewed in Nüsslein-Volhard et al. 1987). Following fertilization, these determinants trigger the expression of the gap gene products, including *hunchback* (*hb*), *Kruppel* (*Kr*) and *knirps* (*kni*), in a series of overlapping domains (Tautz, 1988; Nauber et al. 1988; Stanojevic et al. 1989; Gaul and Jackle, 1987, 1989; Pankratz et al. 1989; Fig. 5A). Subsequently, pair-rule genes are activated in repeating ‘zebra stripe’ patterns, which depend on the gap gene products (Carroll and Scott, 1986; Ingham et al. 1986; Frasch and Levine, 1987; Carroll et al. 1988). These observations together with analyses of partial loss- or gain-of-function mutations of the gap genes (Wieschaus et al. 1984; Lehmann and Nüsslein-Volhard, 1987; Lehmann, 1988; Howard, 1988; Hülskamp et al. 1989; Struhl, 1989a,b) suggest that the overlapping distributions of gap gene proteins provide the spatial cues responsible for pair-rule gene activation. These findings raise the question of how pair-rule genes decode this spatial information into a periodic expression pattern.

There are at least eight pair-rule genes and studies of their interactions suggest that only a few, the ‘primary’ pair-rule genes, respond directly to the positional signals from the gap gene products (Carroll and Scott, 1986; Howard and Ingham, 1986; Frasch and Levine, 1987; Ingham and Gergen, 1988; Carroll et al. 1988). Deletion analyses of two of these primary pair-rule genes, *hairy* and *eve*, suggest that both contain large regulatory domains responsible for generating their periodic patterns of expression which can be subdivided into smaller regions necessary for triggering transcription in specific subsets of stripes (Howard et al. 1988; Goto et al. 1989; Harding et al. 1989; Pankratz et al. 1990). These findings are consistent with the simple hypothesis (Howard et al. 1988) that the control regions of both genes are modular, containing clusters of cis-acting regulatory elements which activate transcription in a single stripe when certain combinations or concentrations of particular gap gene products or maternal determinants are present. The fact that the three gap proteins *hb*, *Kr* and *kni* bind to parts of the control regions of these genes (Stanojevic et al. 1989; Pankratz et al. 1990; Rushlow, personal communication) suggest that this control may be achieved by the direct action of gap proteins.

Here we use gene fusion analysis to assay systematically *hairy* for regulatory elements governing the individual stripe responses. Like the similar, although less exhaustive, analysis of Pankratz et al. (1990) we find that most of the individual stripe responses are
associated with discrete enhancer-like elements. Moreover, we have also been able to dissect some of these regulatory elements into two component parts: one that mediates transcriptional activation in the general vicinity of the stripe, and another that confers local repression of and serves to refine the broad response into a stripe. Thus, our results support the proposal that periodic hairy expression arises piecemeal and suggest that each stripe response reflects the integration of distinct activating and repressing functions operating directly at the level of DNA binding and transcriptional activation.

Materials and methods

Plasmid cloning and transformation were by standard techniques. After establishing individual lines all the flies containing a particular construct were pooled and embryos collected and stained. Constructs that showed staining at the blastoderm stage were identified and several lines were tested individually. Generally, we did not establish homozygous lines and assume that the embryos showing the strongest staining are homozygous for the construct in question.

Fixation was in heptane phase partition with 4% formaldehyde in PEM buffer (100 mM Pipes, pH 6.8; 2 mM EGTA; 1 mM MgSO$_4$). Embryos were devitellinised in 90% methanol, 50 mM EGTA and rinsed 5X in 100% methanol before being rehydrated in TBT (10 mM Tris pH 8.0; 0.25 mM NaCl; 0.1% Triton X-100). Immunohistochemical staining was performed using rabbit anti-beta-galactosidase antibodies (two lots of sera were used, one a generous gift from Paul Macdonald, another a commercial lot from Cappel). Anti-hairy sera was raised in rats using a full-length protein produced in bacteria using the T7 system (Studier and Moffat, 1986). Primary sera were preadsorbed to fixed embryos and used at final dilutions of 1:200 to 1:1000. Signal detection was either by immunofluorescence, in which case fluorochrome-conjugated secondary antisera from either anti-rabbit (Bio Rad) or AP goat anti-rat (Jackson) were used at 1:2000, or by immunochromistry in which case HRP conjugates of goat anti-rabbit (Bio Rad) or AP goat anti-rat (Jackson) were used at 1:2000. All specimens were examined in a Zeiss Axiophot Microscope. Double label fluorescent samples were analyzed using double exposure color, or pairs of single exposure black and white, photomicrographs on Fujichrome 400D at 400 ASA or Ilford XP1 at 400 ASA, respectively. Pairs of images were matched up by printing the entire frame including the borders which were then used to align the two. Immunochromic stains were recorded using Fujichrome 64T at 50 ASA or Technical Pan at 50 ASA developed with HC110.

Results

During cellularization of the blastoderm, the hairy gene is transcribed in a periodic pattern of seven evenly spaced stripes in the middle two thirds of the body (numbered 1–7 in anteroposterior order), as well as in a dorsal patch at the anterior pole (region 0) (Ingham et al. 1985). As described previously (Howard et al. 1988; Rushlow et al. 1989), an upstream regulatory region of at least 15 kilobases is required for expression of stripes 1–7. Moreover, a series of X-ray-induced mutations with breakpoints in this region delete various distal portions of the gene. These mutations eliminate the expression of some but not other stripes indicating that this region includes a series of distinct domains necessary for the formation of particular sets of stripes (Howard et al. 1988). As shown in Fig. 1, these genetic experiments also provide some information about where elements associated with specific responses reside. Proceeding from the distal end (defined by the transformation studies of Rushlow et al. (1989), the mutations $h^{m3}$, $h^{m7}$, $h^{m8}$ and $h^{kl}$ define segments of DNA necessary for expression in stripes 3+4, 6+7, 2, 1+5 and 0, respectively (see Fig. 1).

Although the analysis of these deleted forms of hairy establishes the existence of distinct regulatory elements required for particular stripe responses, it does not provide further insight into how such elements are organized or how they function. For example, it is not clear whether all of the elements necessary and sufficient for a particular stripe response reside together. Nor is it known how such a set of elements could mediate a highly localized transcriptional response in a single position along the body. To gain a better understanding of how periodic hairy expression is generated, we have used an enhancer test vector to assay for regulatory regions necessary and sufficient for activating each stripe response. In brief, parts of the hairy gene were cloned just upstream of a hybrid gene containing a truncated hsp70 heat-shock promoter coupled to the beta-galactosidase structural gene and the resulting constructs introduced into flies by P-element-mediated transformation (Rubin and Spradling, 1982). In this situation, the truncated heat-shock promoter will drive expression of beta-galactosidase if it is activated by a transcriptional control element in the heterologous gene fragment (Lis et al. 1983). For this analysis, we have used the HZ50PL vector developed by Hiromi and Gehring (1987) for their work on the regulation of the pair-rule gene fushi tarazu (fz).

The first construct we tested, ET1, contained a large distal portion of the hairy regulatory domain (Fig. 1) which should contain sequences necessary for expression in stripes 3, 4, 6 and 7, and possibly 2, according to the genetic data outlined above. Immunohistochemical staining of embryos containing this construct for beta-galactosidase protein showed expression in regions apparently corresponding to the ventral parts of stripes 2 and 3 and the whole of stripes 6 and 7 (Fig. 1 and legend). Despite deviations in the relative levels of expression, this initial result indicates that some of the regulatory regions defined previously by deletion analysis contain enhancer-like elements responsible for activating specific stripe responses. This possibility was then examined more systematically by cloning other (smaller) parts of the upstream regulatory into the HZ50PL vector to test for localized transcriptional activation. The different constructs and the resultant expression patterns are diagrammed in Fig. 2. We present these data by considering the minimal sequences necessary for different stripe responses.

Stripes 3 and 4

The only portions of hairy DNA that we have found
expression in the region of stripe 2 not diagramed here (see the discussion of stripe 2). These data define regions of the promoter necessary for expression in particular stripes or groups of stripes. These are summarized by the stippled bars. For instance, \( h^{m8} \) expresses in stripes 1 and 5 whilst \( h^{m7} \) does not. This indicates that DNA between the Aspl site at approximately \(-7\) kb and the EcoRI site at approximately \(-2.5\) kb contains sequence necessary for expression in stripes 1 and 5. For region 0 the mutant and transformation data suggest that the DNA between the Aspl site at approximately \(-5.5\) and the XbaI site at approximately \(+6.5\) is both necessary and sufficient for expression in this region. This is indicated by a filled bar. Finally the part of the 5' region first tested for transcriptional activation in the HZ50 enhancer test assay is shown (ET1). The genetic data show that this DNA contains sequences necessary for expression in stripes 3, 4, 6 and 7, and possibly for stripe 2. (B) Immunochemical staining of ET1. This shows expression of beta-galactosidase antigen in regions corresponding to the ventral parts of stripes 2 and 3 (arrows) and the entire circumferential extent of stripes 6 and 7. The approximate location of these stripes was confirmed by double label immunofluorescence (data not shown). The ventral expression of stripes 2 and 3 may be due to synergy of elements from \( hairy \) with a ventral specific enhancer present in the \( rosy \) gene in the transformation vector (Doyle et al. 1989). The similar construct hSKlacZ of Pankratz et al. (1990) was reported to give a low level of expression in stripe 4. This would be expected if the activity responsible for the weak stripe 4 identified in ET22 (see text) acts at a distance. However, we have not found this to be a consistent feature of ET1 expression in our experiments.

capable of triggering beta-galactosidase expression in the vicinity of \( hairy \) stripes 3 and 4 include a 400 bp segment normally positioned 11.2–11.6 kb upstream from the \( hairy \) transcriptional start site (constructs ET1, 10, 22, 12, 13 and 38). Of these, three (ET10, 13 and 38) have the same proximal end point (–11.2) but different distal endpoints (–15.2, –13.0, and –11.6) and show the following pattern of expression. beta-galactosidase first appears in a broad stripe spanning and including the authentic stripes 3 and 4 (Figs 3A, 4A and 4C). However, in later blastoderms and gastrulae (Fig. 4B) this pattern appears to resolve into a distinct but weak anterior stripe and a broader, stronger posterior stripe. Hence, there appears to be a weak, but correctly localized stripe 3 response and a strong stripe 4 response, which extends too far anteriorly. We cannot
Fig. 2. Summary of hairy-beta-galactosidase constructs. The figure shows a map of hairy very similar to that in Fig. 1, but with more restriction sites: ClaI (C); BglII (G); PvuII (P); SstI (T); SmaI (S); NotI (N). The open bars below this show the pieces of hairy cloned into the HZ50PL vector to create the constructs described in this paper. The number of the construct is shown to the left of the bar, to the right is a summary of the expression pattern observed at blastoderm. A detectable response is shown as a rectangle (the dots are intended to aid visualization and do not indicate expression). The strength of the response is indicated by the degree of shading. For instance, construct ET5 gives a relatively strong response in stripe 1, but a weak one in stripe 5. Where the response is clearly different from the normal stripe this is indicated by elongation of the rectangle: the stripe 4 response of ET10, ET12 and ET38 extends anteriorly; the stripe 5 response of ET2 and ET16 extends posteriorly. Parts of hairy were cloned from lambda phage 6 and 10 of Ish-Horowicz et al. (1985) into pGEM or pSP vectors before transfer to pHZ50PL using either one or two NotI, AspII or blunted ends. Generally there was little variability between different strains and each line seemed to give roughly the same level of expression at the blastoderm stage. However, construct ET22 expressed very weakly and only 1/4 independent lines showed detectable levels of expression in the blastoderm. Note that with the exception of construct ET15 all the hairy fragments are in the normal orientation with respect to the direction of transcription of the heterologous fusion gene.

see any difference between the patterns generated by these three constructs, indicating that all the responsible elements lie in the 400 bp segment in ET38. The construct ET22 contains an additional 400 bp of proximal DNA and shows a different, though related, pattern. In this case, the response in the vicinity of stripe 4 is very much weaker than that in the vicinity of stripe 3 (Fig. 4D and E); however, both the stripe 3 and 4 responses appear to be correctly localized (Fig. 4F). This difference can be attributed to the presence of distinct elements in the 400 bp of proximal DNA present in construct ET22, but absent in ET38, 13 and 10. These results suggest that the stripe 4 response involves at least two discrete components: (i) activation in a broad domain under the control of elements between −11.6 and −11.2 kb, and (ii) repression in an anterior part of this domain mediated by elements located between −11.2 and −10.8 kb. Conversely, all of the elements we have identified associated with a stripe 3 response are contained within the 400 bp segment present in ET38. The data of Pankratz et al. (1990) are consistent with this interpretation: their construct hCClacZ is identical to our ET22 and apparently gives the same pattern, although this is difficult to establish in detail since data showing the stripe 4 response are not presented in their manuscript. Their construct hCRKlacZ apparently also deletes the repressor element and is reported to give fusion of stripes 3 and 4.
Fig. 3. Stripe responses. Double label immunochemistry showing the relationship of the beta-galactosidase (brown) signal to endogenous hairy (blue): (A) ET 38 shows activity in stripes 3 and 4; (B) ET44 shows activity in stripe 6, note the slight posterior displacement of the beta-galactosidase signal; (C) ET15, the same DNA in reverse orientation, shows activity in the region of stripes 2 and 6; (D) ET21 gives a response in stripe 7; (E) ET 17 shows activity only in stripe 1; (F) ET16 shows broad activation in and posterior to stripe 5; (G, H) comparison of the spatial extent of the signals in the stripe 5 region of ET5 and ET16 respectively, the DAB stain was developed to give approximately the same level of signal in both cases. The signal in ET5 (H) is narrower and punctate compared with that in ET16 (G).
Fig. 5. Spatial relationships between \textit{Kr, kni, hairy} and \textit{eve} in the blastoderm.

(A) Double label immunofluorescence showing \textit{Kr} (red) and \textit{kni} (green) in a late cycle 14 blastoderm. The regions of greatest overlap are seen as a region where the signal yellows. Examination of single exposures reveals that the two proteins interpenetrate at least to the centers of their domains; (B) double label immunofluorescence showing \textit{Kr} (red) and \textit{hairy} (green) in a late cycle 14 blastoderm. Note that high levels of \textit{Kr} expression correlate with the activation of \textit{hairy} stripes 3 and 4, whilst lower levels are associated with activation of stripes 2 and 5; note also that high levels of \textit{kni} expression are present only in the vicinity of stripes 4, 5 and 6. (Panel a); (C) double label immunofluorescence showing \textit{eve} (red) and \textit{hairy} (green) in a late cycle 14 blastoderm. Note that in each case the \textit{hairy} stripe overlaps and lies ahead of the \textit{eve} stripe.
Fig. 4. Further analysis of the responses in stripe regions 3 and 4. (A) Immunofluorescence using anti-beta-galactosidase of an ET°0 embryo showing the broad response in the region of stripes 3 and 4; (B) during gastrulation a distinct anterior patch of expression (arrow) can be seen (p marks the posterior end of the germ band); (C) Double label immunofluorescence showing the relationship of the beta-galactosidase signal (bottom half of the figure) to the endogenous hairy signal (top half of the figure) in the same embryo shown in panel A; (D) Surface and (F) medial focal views of immunofluorescence to detect beta-galactosidase in an ET22 embryo showing the weak response in the region of stripe 3 and the very low level of expression in parts of stripe 4 (arrow); (F) Double label immunofluorescence showing the relationship of the beta-galactosidase signal (bottom half of the figure) to the endogenous hairy signal (top half of the figure) in the same embryo shown in D and E. The double label montages were prepared as described in Materials and methods.

although the changes leading to this fusion are not documented, leaving open the possibility that a different explanation applies.

Stripe 7
Comparison of constructions ET22, 21 and 29° indicates that a 1.4 kb region of DNA (between positions -11.2 and -9.8 kb) contains elements necessary and sufficient to trigger correctly localized expression in stripe 7 (as shown in Fig. 3D). An attempt (ET29) to refine further the location of the responding elements by deleting a distal 400 bp portion of this region (between -11.2 and -10.8 kb) failed, indicating that it contains some elements which are necessary, though not sufficient (ET22), for a stripe 7 response. Note that this same 400 bp segment seems to contain elements mediating local repression of the stripe 4 response (see above). Thus, at the present level of analysis, elements mediating the stripe 4 response appear to overlap with those mediating both stripe 3 and 7, though the latter two responses are governed by elements that can be cleanly separated from each other.

Stripes 2 and 6
The initial construct ET1 includes DNA capable of conferring beta-galactosidase expression in the vicinity
of the endogenous stripe 6 as well as weak and partial expression in the vicinity of stripe 2. Further deletion analysis of this region (particularly constructs ET44, 30 and 31) provides clear evidence that an 600 bp region of DNA (from position -8.4 to -9.0; ET31) contains all of the elements necessary and sufficient for a relatively normal stripe 6 response (Fig. 3B). However, we note that this response seems to be displaced slightly posterior to the location of the endogenous stripe 6 at this stage.

Based on the results presented so far, we considered it likely that these same constructs would give a stripe 2 response. They did not. However, we were able fortuitously to uncover a stripe 2 response by testing one of the DNA fragments used to define the stripe 6 response (construct ET44) in the reverse orientation relative to the hsp70 promoter. This construct (ET15) shows a normal stripe 6 response, indicating that the elements that mediate this response do so in an orientation-independent fashion. In addition, it also triggered a response in the region of stripe 2 (Fig. 3C). We do not understand this effect, but note that the mutations h^m7 and h^m8 give weak responses in vicinity of stripe 2 as does the ET1 construct, consistent with the notion that at least two separate stripe 2 elements are present in the middle portion of the hairy upstream regulatory region. It is difficult to compare these results with those of Pankratz et al. (1990). They also see a stripe 6 activity in this region with their construct hRKlacZ but their stripe 2 response seems to be different from ours.

Stripes 1 and 5
As diagrammed in Fig. 2, the analysis of constructs including DNAs spanning the region from -9.6 to -0.4 kb (ET 2,16,5,17,8,6 and 7) reveal the presence of distinct elements necessary and sufficient for the remaining stripe 1 and 5 responses. Elements necessary and sufficient for triggering the stripe 1 response reside in a 1.0 kb DNA fragment between positions -5.4 and -4.4 (construct ET17; Fig. 3E). However, the stripe 5 response appears more complex, depending, as is the case for the stripe 4 response, on distinct elements mediating local activation and local repression. Constructs ET2 and 16 both contain DNA between positions -9.6 and -5.4, but do not contain more proximal sequences; both confer a broad stripe response, which differs from the normal stripe 5 in extending more much posteriorly (past stripe 6; see Fig. 3F). However, construct ET5, which differs from ET16 only in that it includes an additional 1.0 kb of more proximal DNA, produces a different response in the vicinity of stripe 5 (Fig. 3H). In this case, the response is relatively weak and occurs only in some of the nuclei within the normal stripe 5 region. To confirm that this difference was not simply due to changes in level of expression, we reduced the length of time that the peroxidase reaction was developed for some ET16 embryos so that the signal was comparable to that of a batch of ET5 embryos. As shown in Fig. 3 panels G and H, this experiment demonstrates that inclusion of the additional proximal DNA has altered the spatial properties of the response. Thus, DNA sequences between -5.4 and -4.4 kb are involved both in reducing the level of stripe 5 expression and in completely repressing inappropriate expression posterior to the endogenous stripe. One interpretation of this result is that the additional proximal sequences contain a distinct element that antagonizes activation mediated by other elements present in more distal portions of DNA (i.e. between -8.1 and -5.4; ET5) and that this element responds to a different spatial signal thereby defining the posterior boundary of stripe 5. The elements mediating repression reside in the same 1.0 kb interval of DNA containing all of the elements necessary and sufficient for triggering the stripe 1 response (ET17); hence, the two sets of elements may overlap.

Region 0
We failed to observe activation of the truncated hsp70 promoter in region 0 in any of our constructs (see Fig. 2). Similarly, a hairy-beta-galactosidase fusion gene extending proximally from -4.2 kb through the hairy promoter and most of the structural gene (to position +2.5 kb) also failed to activate transcription in this region. Finally, we note that a P-element-beta-galactosidase fusion gene inserted in the upstream regulatory region of the hairy gene (at position ~-1 kb) is expressed in stripes 1-7, but not in region 0 (Fusano et al. 1989). Thus, our only evidence for a discrete region associated with this response remains the genetic data indicating that region 0 expression depends on DNA downstream from the breakpoint of the h^kl mutation (see Fig. 1).

Discussion
In prior genetic experiments, Howard et al. (1988) established that the large upstream regulatory region of the hairy gene contains distinct segments of DNA necessary for subsets of the initial eight stripes of expression. Here, we confirm and extend these findings by defining unique portions of this upstream DNA that are both necessary and sufficient for triggering each of the stripe responses. This analysis and the similar work of Pankratz et al. (1990) both provide strong support for the proposal (Howard et al. 1988) that periodic hairy expression arises as a series of singular stripe responses each mediated by different combinations or concentrations of regulatory factors such as the gap gene products. In addition, dissection of the regulatory elements governing at least two of these responses (stripes 4 and 5) suggests that each response is achieved by integrating distinct activating and repressing signals.

Unique subsets of cis-acting regulatory elements mediate different hairy stripe responses
With the exceptions of stripes 0 and 2, our analysis has allowed the identification of relatively small segments of upstream regulatory DNA capable of mediating each
of the individual stripe responses constituting periodic *hairy* expression. In each case, these portions of DNA behave like cis-acting enhancer elements capable of triggering transcription of a heterologous promoter in domains that coincide with a particular endogenous *hairy* stripe. In general, we were not able to separate cleanly the regulatory sequences controlling different stripe responses – the minimal segments of DNA necessary for controlling particular stripe responses (e.g. 4) often seem to include sequences relevant to the regulation of one or more other responses (e.g. 3 and 7). Furthermore, we have some evidence suggesting that two separate regions may play a role in generating the stripe 2 response (one associated with construct ET15 and another more proximal region defined by the breakpoint of the *h^mb* mutation; see Figs 1, 2). Nevertheless, we have found that the different stripe responses can be produced by unique, albeit overlapping, segments of DNA. We therefore conclude that each stripe response depends on a distinct combination of cis-acting regulatory sequences, and hence, by extension, on different combinations or concentrations of regulatory factors. That the sequences governing two or more stripe responses often appear to be interspersed or overlapped suggests the possibility that the same regulatory factors may be involved in regulating different stripe responses via common target sites on the DNA.

Generating precise stripe responses by integrating local activation and repression

Several lines of evidence indicate that overlapping, graded distributions of gap gene products (e.g. Fig. 5) play a critical role in controlling periodic *hairy* expression. Most significantly, reduction or loss of function of each gap gene abolishes expression of particular *hairy* stripes whilst others change size and position, generally expanding and moving towards the gap left by the absence of the missing stripe(s) (Ingham et al. 1986; Howard, 1988; Ingham and Gergen, 1988; Carroll and Vavra, 1989; Hooper et al. 1989; Pankratz et al. 1990). Moreover, the apparent concentrations of gap proteins appear to peak in the vicinity of the *hairy* stripes eliminated in mutant embryos and to decline progressively in the regions where the stripes are correspondingly shifted (ibid). Taken together, these results suggest that some *hairy* stripes are activated in response to critical threshold concentrations of particular gap gene products, whilst others may be repressed in response to different threshold concentrations.

Although our data argue that local activation is the primary mode of *hairy* regulation, they also indicate that local repression plays a significant role. Most tellingly, we find that removal of a particular control element can both relax the precision of a given stripe response as well as increase the level of expression. For example, when part of the minimal segment conferring the stripe 5 response is deleted, expression both increases and spreads significantly posterior to the normal posterior boundary. Similar results have also been observed with the DNA intervals controlling the stripe 4 and 1 responses (Figs 2 and 4, and our unpublished findings). Hence, the precise position, width and intensity of a given *hairy* stripe may reflect modulation of a relatively crude activation response by a variety of spatially localized repressing functions.

The possibility that each *hairy* stripe response depends on integrating both activating and repressing signals raises the question of how factors mediating local repression of one stripe avoid interfering with the spatial regulation of adjacent stripes. It is clear from our analysis that the regulatory elements mediating the response to the activators behave like enhancers, capable of driving expression of the *hairy* or *hsp70* promoter at a remove of several kilobases. It is also clear that closely linked sequences mediate partial repression of these enhancer elements without affecting activation events mediated by enhancers positioned a few kilobases away. For example, stripe 5 appears to be generated by at least two components: activation in a broad domain that will encompass both stripe 5 and stripe 6, and repression in a posterior portion of this domain including where stripe 6 will form. Yet, stripe 6 is apparently not affected by the process of local repression that governs the stripe 5 response. We suggest that this autonomy between neighboring stripe responses arises because the factors causing repression act only locally at the DNA level to prevent activation mediated by the stripe-specific enhancer sequences. Many of the proposed mechanisms for transcriptional repression involve masking of sites on the DNA that bind positive activators, or of sites on these activators that interact with other transcription factors to initiate transcription (reviewed in Levine and Manley, 1989; Renkawitz, 1990). Such mechanisms would require local interactions between repressors and activators at the level of the DNA and could account for the ability of repressors to block the function of one enhancer (e.g. stripe 5) without affecting activation mediated by another enhancer (stripe 6) positioned a few kilobases away. Perhaps the degree of modularity exhibited by the *hairy* promoter reflects the role of local interactions between repressors and activators in controlling particular stripe responses.

Recently, Carroll and Vavra (1989; see also Carroll, 1990) have interpreted data on the effects of inhibitors of protein synthesis (Edgar et al. 1986, 1989) and the pattern of expression of *hairy* in various gap mutant combinations (Howard, 1988; Ingham and Gergen, 1988; Carroll and Vavra, 1989; Hooper et al. 1989) to suggest that *hairy* stripes are established by local repression in interstripe regions. Such a mechanism is not readily compatible with our finding that most of the *hairy* stripe responses can be attributed to stripe-specific enhancers mediating transcriptional activation. We argue instead that effects of protein synthesis inhibitors and various combinations of gap gene mutations on periodic *hairy* expression reflect a secondary role of local repression in refining initially crude domains of gene activation driven by the stripe-specific enhancers.
A decoding role for the hairy upstream regulatory region

The role of the upstream regulatory region of hairy in generating periodic gene expression contrasts markedly with that of the ftz gene, which has been extensively analyzed by similar methods. In the latter instance, a relatively small 'zebra' element had been identified that appears to contain a set of general activation elements as well as a set of elements mediating repression by hairy and possibly other regulatory factors (Hiromi et al. 1985; Howard and Ingham, 1986; Hiromi and Gehring, 1987; Ish-Horowicz and Pinchin, 1987; Dearolf et al. 1989a,b). Thus, the upstream regulatory region of hairy may execute the primary role of the gene by functioning as a complex receptor that decodes the prepattern of gap gene protein distributions into a simpler periodic signal. This signal is then used to generate the zebra patterns of secondary pair-rule gene products like ftz, which define the boundaries of parasegments and organize pattern within these units.

hairy and eve and the generation of segmental polarity

Recent analyses of the pair-rule gene eve suggest that its periodic expression may be generated in a similar fashion to that of hairy. Periodic eve expression appears to be altered in comparable ways to that of hairy in response to various gap gene mutations (Frasch and Levine, 1987; Carroll et al. 1988). Moreover, eve seems to have a similar promoter structure containing discrete stripe-specific control elements (Goto et al. 1989; Harding et al. 1989), although these elements differ from the hairy elements in that they are less effective in mediating local activation of the heterologous hsp70 promoter.

Comparison of the eve and hairy stripe responses indicates that they are almost coincident except that the eve stripes are invariably shifted one to two cells anteriorly to the hairy stripes (Carroll et al. 1988; Hooper et al. 1989; Fig. 5). This consistent displacement provides the first indication of segmental polarity. Moreover, given the complex regulatory interactions known to occur between the various pair-rule genes (Carroll and Scott, 1986; Harding et al. 1986; Howard and Ingham, 1986; Frasch and Levine, 1987; Hiromi and Gehring, 1987; Carroll et al. 1988; Frasch et al. 1988; Ingham and Gergen, 1988; Lawrence and Johnston, 1989), as well as the roles of these genes in controlling the initial expression of segment polarity genes such as wingless and engrailed (Howard and Ingham, 1986; DiNardo and O'Farrell, 1987; Lawrence et al. 1987; DiNardo et al. 1988; Ingham et al. 1988; Martinez-Arias and White, 1988), it is clear that this displacement could serve as an initial cue that confers segmental polarity. Hence, a slight but consistent difference in the way gap gene products activate the corresponding stripe responses of eve and hairy may play a critical role in generating polarity. Such a skew could result from slightly different affinities of their cis-acting elements for these regulatory factors or from a more significant difference in the way these elements mediate transcriptional activation of the two genes.

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late periodic patterns of even-skipped expression are controlled by distinct regulatory elements that respond to different spatial cues. Cell 57, 413–422.


