Short-range transcriptional repressors mediate both quenching and direct repression within complex loci in *Drosophila*

Susan Gray and Michael Levine

Department of Biology, Center for Molecular Genetics, University of California at San Diego, La Jolla, California 92093-0347 USA

The early *Drosophila* embryo provides a unique system for the analysis of transcriptional repression since a broad spectrum of repressors are distributed in spatially distinct patterns. Krüppel (Kr) and snail (sna), two zinc finger repressors, are essential for segmentation and for the establishment of the mesoderm/neuroectoderm boundary, respectively. Both repressors were examined in the context of synthetic gene complexes containing modular promoters and divergently transcribed reporter genes. These studies indicate that Kr and sna function as short-range repressors, which can mediate either quenching or direct repression of the transcription complex, depending on the location of repressor sites. When located within an upstream enhancer, the repressor locally quenches nearby activators and permits other enhancers to interact with the transcription complex (enhancer autonomy). In contrast, when bound to promoter-proximal regions the repressor functions in a dominant fashion and blocks multiple enhancers. Local quenching and dominant repression require close linkage (<100 bp) of the repressor with either upstream activators or the transcription complex. These studies establish short-range repression as a flexible form of gene regulation and suggest that the key distinction among repressors is their range of action.

[Key Words: *Drosophila* embryo; transcriptional repression; zinc finger repressors; Krüppel; snail; gene regulation]

Received December 18, 1995; revised version accepted February 1, 1996.

Previous studies have demonstrated the importance of transcriptional repression in establishing cell type-specific patterns of gene expression. The yeast $\alpha 2$ homeo domain protein specifies the α -cell phenotype by repressing α -specific genes (Keleher et al. 1992). In the *Drosophila* embryo, stripes of segmentation gene expression are established by repressors that define the borders of individual stripes (for review, see Pankratz and Jackle 1990; Small and Levine 1991). Repression is also essential for establishing the boundary between the presumptive mesoderm and neuroectoderm (Alberga et al. 1991; Kosman et al. 1991; Leptin 1991). In addition, repressors both define and maintain the expression limits of homeotic selector genes (Paro 1993; Busturia and Bienz 1993; Chan et al. 1994). Numerous examples of transcriptional repression have been observed in mammalian systems. For example, repression establishes the rhombomere-specific pattern of Hoxb-1 expression within the hindbrain region of the mouse embryo (Studer et al. 1994). Repression is also essential for B-cell specific expression of the immunoglobulin heavy-chain enhancer (Genetta et al. 1994) and the regulation of CD4 expression in different T-cell lineages (Sawada et al. 1994; Siu et al. 1994).

Several different modes of repression have been proposed, including competition, quenching, and direct repression of the transcription complex (for review, see Levine and Manley 1989; Johnson 1995). The yeast $\alpha 2$ homeo domain protein represents the most thoroughly characterized eukaryotic repressor. Occupancy of $\alpha 2$ operator sites depends on cooperative DNA-binding interactions with a general transcriptional activator, Mcm1 (Vershon and Johnson 1993). Once bound, $\alpha 2$ recruits the Tup1 repressor, which directly inhibits the transcription complex over distances of 200 bp to 1 kb (Keleher et al. 1992; Herschbach et al. 1994; Cooper et al. 1994). It is unclear whether Tup1 also works in a local fashion to inhibit (quench) the neighboring Mcm1 activator.

In vitro transcription experiments and transient transfection assays suggest that a number of mammalian repressors, including nuclear receptor proteins such as the glucocorticoid and thyroid hormone receptors, might directly repress the transcription complex (Stromstedt et al. 1991; Drouin et al. 1993; Fondell et al. 1993). However, in many instances repressor binding sites are contained within composite *cis* regulatory elements that map far (>200 bp) from the transcription start site (Pearce and Yamamoto 1993). It is conceivable that such

repressors mediate both local quenching of upstream activators and direct repression of the transcription complex. To address this issue we have analyzed the activities of two *Drosophila* zinc finger repressors, *snail* (*sna*; Boulay et al. 1987) and Krüppel (*Kr*; Gaul and Jackle 1987).

sna is directly activated by the maternal dorsal (*dl*) regulatory gradient (Ip et al. 1992a) and is expressed specifically in ventral regions where it defines the limits of the presumptive mesoderm (Ip et al. 1994). *sna* represses the expression of *rhomboid* (*rho*) in the mesoderm and thereby restricts its expression to lateral stripes in the presumptive neuroectoderm (Ip et al. 1992b). *Kr* is regulated by the bicoid (*bcd*) maternal gradient (Hoch et al. 1992); its expression is restricted to central regions of the embryo corresponding to the presumptive thorax and anterior abdomen (Gaul and Jackle 1987). *Kr* helps establish pair-rule stripes of gene expression, at least in part, by functioning as a repressor (Zuo et al. 1991; Licht et al. 1993; Sauer et al. 1995). For example, it represses the *even-skipped* (*eve*) stripe 2 enhancer and thereby defines the posterior stripe border (Stanojevic et al. 1991; Small et al. 1991, 1992).

Recent studies suggest that *sna* represses transcription through a short-range quenching mechanism (Gray et al. 1994). A 300-bp enhancer is responsible for regulating lateral stripes of *rho* expression in the early embryo. This enhancer contains high-affinity *dl* sites, as well as basic-helix-loop-helix (bHLH) activator sites; these work synergistically to activate *rho* in both ventral and lateral regions (Ip et al. 1992b). The enhancer also contains four closely linked *sna* repressor sites that exclude expression from the ventral mesoderm. Two of these sites directly overlap activator sites (Ip et al. 1992b), suggesting that *sna* might function through a competition mechanism. However, modified *rho* enhancers were subsequently shown to direct essentially normal patterns of expression even when *sna* repressor sites were moved 50–75 bp away from the closest activators. These observations prompted the proposal that *sna* may work over short distances to quench nearby activators through local protein-protein interactions (Gray et al. 1994).

Here, we present evidence that *Kr* can also function through a short-range quenching mechanism, as suggested by previous tissue culture assays (Zuo et al. 1991). This observation challenges previous proposals that *Kr* defines the posterior border of *eve* stripe 2 through competition (Stanojevic et al. 1991; Small et al. 1991, 1992). Moreover, *sna* and *Kr* are shown to function in promoter-proximal regions of test promoters, presumably through a direct repression mechanism. The range of repression is similar for quenching and direct repression; that is, *sna* and *Kr* must bind near (within 100 bp) upstream activators or the transcription start site. The location of the repressor sites, within upstream enhancers or promoter-proximal regions, determines distinct patterns of gene expression. When located within an upstream enhancer, the repressor functions in a local fashion and permits other enhancers to make contact with the transcription complex (enhancer autonomy). In con-

trast, the same repressor functions in a dominant fashion and blocks multiple enhancers, when located near the transcription start site. These results suggest that a common repressor can mediate both quenching and direct repression of the transcription complex and that short-range repression represents a flexible form of gene regulation.

Results

Efficient ventral repression of the *rho* neuroectoderm enhancer element (NEE) requires close proximity of *sna* and *dl* binding sites (Gray et al. 1994). *sna* was also found to inhibit the heterologous *bcd* activator when bound within the *eve* stripe 2 enhancer or a synthetic enhancer containing *bcd* and *dl* activator sites. These findings prompted the proposal that short-range repression should allow enhancers to function autonomously within complex, modular promoters (Gray et al. 1994). An example of a synthetic modular promoter is shown in Figure 1A. A nearly additive staining pattern (lateral and transverse stripes) is observed when a minimal 152-bp *rho* NEE is placed upstream of the 480-bp *eve* stripe 2 enhancer. *sna* bound to the *rho* NEE represses *dl*/bHLH activation in the presumptive mesoderm but does not interfere with ventral activation of the neighboring stripe 2 enhancer. The most closely linked *sna* and *bcd* sites are separated by ~175 bp, beyond the range of efficient *sna* repression [see diagram below Fig. 1A and summary in Fig. 1B].

The *Kr* repressor also appears to function locally, within the limits of the *eve* stripe 2 enhancer (Fig. 1B). However, there is a slight attenuation of the *rho* lateral stripes in central regions containing peak levels of *Kr* protein (arrows, Fig. 1A). Subsequent studies (see Fig. 5) suggest that the *Kr* site at the 3' end of the stripe 2 enhancer, located just 45 bp from the transcription start site, mediates direct repression of the transcription complex. This repression is relatively weak, as the *bcd* activator and *Kr* repressor compete for overlapping binding sites (Small et al. 1991, 1992; see below).

Kr appears to mediate direct repression of the transcription complex

Kr can repress a defective 700-bp *rho* NEE that lacks all four *sna* repressor sites (NEE Δ *sna*). As shown previously (Ip et al. 1992b), removal of the *sna* site causes a loss of ventral repression, so that there is equal expression in both ventral and lateral regions (Fig. 2A). In these experiments, the NEE Δ *sna* was placed between two divergently transcribed promoters, a leftward *white* reporter gene and a rightward *lacZ* gene. The expression of either gene can be assayed independently by *in situ* hybridization using digoxigenin-labeled *white* or *lacZ* antisense RNA probes (Tautz and Pfeifle 1989; Cai and Levine 1995).

Kr-binding sites positioned 50 bp upstream and 55 bp downstream of the nearest *dl* activator sites result in an almost complete loss of staining in central regions where

Figure 1. Enhancer autonomy in a modular promoter. The transgenic embryo is oriented with anterior to the *left* and dorsal *up*. It contains a fusion promoter with a minimal, 152-bp *rho* NEE placed upstream of the 480-bp *eve* stripe 2 enhancer (see summary diagram below the embryo). The expression pattern was visualized after hybridization with a digoxigenin-labeled *lacZ* antisense RNA probe. (A) Embryo at the midpoint of nuclear cleavage cycle 14. The staining pattern consists of *rho* lateral stripes and the transverse *eve* stripe. The stripe 2 enhancer contains five *bcd* activator sites and three *Kr* repressor sites, whereas the *rho* NEE contains two *dl* activator sites and three *sna* repressor sites. The most closely linked *rho* NEE repressor site and stripe 2 activator are separated by 175 bp. (B) Summary of short-range repression and enhancer autonomy. The staining pattern obtained with the fusion promoter is additive, as repressors bound to one enhancer do not affect the neighboring enhancer. According to this diagram, the *sna* repressor functions in a local fashion to inhibit, or quench, nearby activators in the *rho* NEE. Activators in the neighboring stripe 2 enhancer map beyond the range of *sna* repression (~50–100 bp). Similarly, stripe 2 repressors, such as *Kr*, do not interfere with the expression of the NEE.

there are high concentrations of *Kr* protein (Fig. 2A). The simplest explanation for this observation is that *Kr* locally quenches or inhibits nearby *dl* activators. This is supported by the observation that *white* is also repressed by *Kr* (Fig. 2B), even though the nearest repressor site maps >550 bp from the *white* initiation site (see diagram below Fig. 2A). Previous tissue culture assays are also consistent with the notion that *Kr* functions via quenching (Zuo et al. 1991).

Further evidence for short-range quenching was obtained by moving the *Kr* sites away from the *dl* activators. When positioned ~150 bp from *dl*, *Kr* no longer represses *white* (Fig. 2D). This result is consistent with the notion that *Kr* must map within ~100 bp of upstream activators to inhibit expression, similar to the situation shown previously for *sna* (Gray et al. 1994).

However, we were surprised that this arrangement of *Kr* sites resulted in substantial repression of *lacZ* (Fig. 2C). The simplest explanation is that the proximal *Kr* site, located within 75 bp of the *lacZ* transcription start site, mediates direct repression of the transcription complex (see below). A number of additional experiments were done to determine whether *Kr* and *sna* can mediate both local quenching of upstream activators and direct repression of the transcription complex.

sna can repress the transcription complex

Our previous analysis of *sna* repression did not include a critical test for direct repression (Gray et al. 1994). *sna* sites located ~50 bp from *dl* activators restore ventral repression to an otherwise defective NEE Δ *sna* (Fig. 3A). This arrangement of *sna* sites results in efficient repression of both the rightward *lacZ* gene (Fig. 3A) and the leftward *white* gene (Fig. 3B). Repression is probably caused by local quenching of *dl* activators, as moving the *sna* sites another 100 bp results in a near loss of *sna*-mediated repression (Gray et al. 1994). Moreover, *sna* represses *white* expression even though the closest *sna* site maps 550 bp from the *white* initiator (Fig. 3B).

To determine whether *sna* can directly repress the transcription complex, two *sna*-binding sites were placed ~50 bp upstream of the *lacZ* transcription start site. This results in efficient repression of the *lacZ* pattern in ventral regions (Fig. 3C). In contrast, *white* is depressed completely in ventral regions (Fig. 3D), suggesting that *sna* does not interfere locally with *dl* but, instead, represses the *lacZ* promoter (the *sna* sites map 215 bp from the nearest *dl* activator site in the NEE Δ *sna*).

Further evidence that *sna* can mediate direct repression was obtained by placing the NEE Δ *sna* 3' of *lacZ* (Fig. 4). The insertion of two *sna* sites ~50 bp upstream of the *lacZ* start site restores an essentially normal pattern (Fig. 4A). Repression is almost certainly caused by direct repression of the transcription complex, as the nearest NEE Δ *sna* activators now map >4.5 kb from *sna*. Moving the *sna* sites another 75 bp upstream of *lacZ* abolishes repression (Fig. 4B). These results suggest that *sna* can repress the transcription complex only over short distances, similar to the range observed for *sna*-mediated quenching of upstream activators.

Dominant repression of multiple enhancers

As discussed earlier, short-range repressors bound to one enhancer do not interfere with neighboring enhancers in a modular promoter (e.g., Fig. 1A). The next series of experiments examined how the activity of multiple enhancers might be affected by the placement of *sna* and *Kr* repressor sites near the transcription start site.

Previous studies identified a 260-bp enhancer (PE) from the *twist* (*twi*) promoter region that directs expression in the ventral-most 12–14 cells in response to peak concentrations of the *dl* regulatory gradient (Jiang et al. 1991; Pan et al. 1991; Thisse et al. 1991). Two tandem

Figure 2. Kr can mediate short-range quenching of heterologous activators. Transgenic embryos are oriented as described in Fig. 1. Modified *rho* NEE sequences were inserted between two divergently transcribed reporter genes, *white* and *lacZ*, which can be visualized separately following in situ hybridization. These 700-bp *rho* NEEs lack native *sna* repressor sites (NEE Δ *sna*) and contain synthetic Kr sites. (A) *lacZ* staining pattern of a precellular embryo containing the NEE Δ *sna* enhancer with Kr-binding sites located ~50 bp from dl-activator sites. Efficient repression is observed in central regions where there are high concentrations of Kr protein. The Kr sites map far from the *white* and *lacZ* transcription start sites (550 and 180 bp, respectively; see summary diagram below the embryo). (B) Gastrulating embryo containing the same fusion gene as in A, except that the *white* reporter gene is visualized. Efficient repression is observed in central regions. (C) *lacZ* staining pattern of a precellular embryo containing the NEE Δ *sna* with more distantly linked Kr repressor sites. These sites map ~150 bp from the nearest dl activator sites. Repression is nearly as efficient as that observed with more closely linked Kr sites (cf. A). (D) Gastrulating embryo containing the same fusion gene as in C except that *white* expression is visualized. No repression is observed in central regions.

copies of this enhancer (2XPE) direct a somewhat more robust staining pattern than a single copy (Jiang and Levine 1993). A fusion promoter containing 2XPE placed upstream of the *rho* NEE directs an additive staining pattern, which consists of *rho* lateral stripes and a band of expression within the presumptive mesoderm (Fig. 5A; arrow indicates the gap between the expression patterns directed by the two enhancers). A single Kr-binding site placed between the two enhancers, >150 bp from activators in either the 2XPE or *rho* NEE, has no discernible effect on the pattern (Fig. 5A). In contrast, both enhancers are repressed when the same Kr site is placed ~75 bp from the transcription start site (Fig. 5B). The *rho* NEE is attenuated in central regions, whereas 2XPE expression is nearly abolished.

sna was also tested for its ability to repress multiple enhancers when placed near the transcription start site. Figure 6A shows the staining pattern obtained with a fusion promoter containing the 480-bp *eve* stripe 2 enhancer placed upstream of a 500-bp stripe 3 enhancer. The two enhancers are separated by a 160-bp neutral spacer sequence from the native *eve* promoter region (Small et al. 1993). An additive staining pattern is observed, although stripe 2 expression is slightly weaker

than stripe 3 staining (Fig. 6A). A variable head stripe results from cryptic regulatory sequences within the P-transformation vector (Jiang et al. 1991; Small et al. 1992). In addition, stripe 7 expression is mediated by regulatory sequences located primarily in the stripe 3 enhancer (Small et al. 1993, 1996). A cluster of four *sna*-repressor sites has no effect on the ventral expression of either stripe 2 or stripe 3 when located in the spacer region. These map far (>150 bp) from the closest activators in either enhancer.

Stripe 2 is repressed in ventral regions when several *sna* sites are inserted within the stripe 2 enhancer (Fig. 6B). These *sna* sites map ~30–40 bp from critical *bcd* sites within the modified enhancer. Stripe 3 is unaffected, as the stripe 3 enhancer is located beyond the range of *sna* repression. The insertion of *sna* repressor sites just upstream of the *lacZ* initiation site results in the ventral repression of both stripes (Fig. 6C). The stripe 2 pattern is somewhat more efficiently repressed than stripe 3; the stripe 2 enhancer may be inherently weaker than the stripe 3 enhancer (see Cai and Levine 1995). Nonetheless, these results suggest that *sna* can function as a dominant repressor to block multiple enhancers when bound near the transcription complex.

Figure 3. The *sna* repressor can silence the transcription complex. Transgenic embryos are oriented as described previously. They contain the 700-bp NEE Δ *sna* enhancer between divergently transcribed *white* and *lacZ* reporter genes. (A) *lacZ* staining pattern in a precellular embryo with synthetic *sna* sites placed $\sim$ 50 bp from *dl* activator sites (see diagram beneath the embryo). Efficient repression is observed in ventral regions where there are high concentrations of *sna* protein. (B) Gastrulating embryo expressing the same fusion gene as A except that *white* expression is monitored. Repression is observed in the presumptive mesoderm. (C) *lacZ* staining pattern in a precellular embryo obtained with two *sna* sites placed $\sim$ 50 bp upstream of the *lacZ* transcription start site. These mediate efficient repression in the presumptive mesoderm. (D) Gastrulating embryo expressing the same fusion gene as C except that *white* expression is monitored. The *sna* sites located near *lacZ* fail to repress *white* expression. Consequently, the staining pattern is derepressed in ventral regions.

The *sna* repression domain can be uncoupled from DNA binding

Previous studies have established that transcriptional activators possess distinct DNA-binding domains and activation domains (e.g., Chi et al. 1995). Repressors have not been characterized to the same extent, although recent tissue culture assays have identified discrete repression domains (Han and Manley 1993a,b; Gerwin et al. 1994; Licht et al. 1994). To determine whether the non-DNA-binding domain of *sna* is sufficient for repression in vivo, we analyzed the activities of a *sna*-Gal4 fusion protein in transgenic embryos.

A 200-bp *rho* NEE was modified extensively to make it responsive to the fusion protein (see Materials and methods). The modified NEE contains three *dl*, three *twi*, and three Gal4 upstream activating sequence (UAS) binding sites (see diagram below Fig. 7A) and directs strong expression in ventral and ventrolateral regions, encompassing $\sim$ 20–22 cells (Fig. 7A).

The *sna*-Gal4 fusion protein consists of the non-DNA-binding domain of *sna* fused to the DNA-binding domain of the yeast activator Gal4. *sna* is composed of 390-amino acid residues; the carboxy-terminal sequences lo-

cated between amino acids 245 and 390 contain five zinc fingers that mediate DNA binding (Boulay et al. 1987; Ip et al. 1992b; Mauhin et al. 1993). *sna* coding sequences for amino acids 1–244 were placed upstream of the Gal4 amino acid 2–93 coding region. The fusion gene was placed under the control of a chimeric promoter containing the 2XPE *twi* enhancer attached to a 1.6-kb sequence from the *sna* promoter region. As shown previously, this *twi*-*sna* chimeric promoter directs expression in the ventral-most 14–16 cells (Ip et al. 1994). The modified NEE was introduced into transgenic embryos expressing the *sna*-Gal4 fusion gene (Fig. 7B). The fusion protein mediates efficient repression in the ventral-most 14–16 cells, thereby converting the wide band of ventrolateral staining into two narrow stripes (cf. Fig. 7B and 7A).

The repression mediated by the *sna*-Gal4 fusion protein is probably caused by short-range inhibition of nearby *dl* activators, rather than direct repression of the transcription complex. The proximal-most Gal4 site is located >100 bp from the *lacZ* transcription start site. A competition mechanism of repression is also unlikely, as Gal4 fusion proteins that activate gene expression do not repress the modified NEE. For example, the modified NEE is activated in anterior regions of transgenic em-

Figure 4. *sna* can silence a *rho* NEE located far from the transcription start site. Precellular, transgenic embryos are oriented as described previously. Both embryos contain a 700-bp *rho* NEE Δ *sna* downstream of the *lacZ* gene. (A) *lacZ* staining pattern obtained when two *sna* sites are placed ~50 bp from the transcription start site (see diagram beneath the embryo). Efficient repression is observed in the presumptive mesoderm. (B) Same as A except that the *sna* sites were moved another 75 bp from the *lacZ* start site. Little or no repression is observed, so that the staining pattern is derepressed completely in ventral regions. Different transgenic lines containing the fusion gene in A exhibit somewhat variable staining patterns. The staining pattern in A represents efficient ventral repression; other lines show slightly derepressed patterns (data not shown).

bryos containing an anteroposterior gradient of a bcd–Gal4 fusion protein (Simpson-Brose et al. 1994; data not shown).

Discussion

We have shown that short-range repression represents a flexible form of gene regulation. Binding of repressor to remote locations in the promoter region results in local inhibition, or quenching, of upstream activators. Other enhancers contained in the promoter are not influenced by the repressor and can interact freely with the transcription complex. In contrast, the binding of repressor to promoter–proximal regions results in the dominant repression of the promoter, so that multiple enhancers are blocked. This flexibility contrasts with long-range repressors (summarized in Fig. 8), which can block mul-

multiple enhancers over distances of several kilobases regardless of location within a gene complex (Huang et al. 1993; Jiang et al. 1993; Kirov et al. 1993; Lehming et al. 1994). We propose that the most crucial distinction among repressors is their range of action.

Short-range vs. long-range repression

Previous studies have identified several apparently distinct modes of repression, including competition, quenching, and direct repression of the transcription complex (for review, see Levine and Manley 1989; Johnson 1995). Evidence for competition stems from the organization of enhancers and promoter elements active in the early embryo. For example, four of the six repres-

Figure 5. The Kr repressor can silence multiple enhancers. Precellular transgenic embryos are oriented as in Figs. 1–4. They express a synthetic modular promoter containing the 2XPE mesoderm-specific enhancer placed upstream of the *rho* NEE. (A) *lacZ* staining pattern obtained with the fusion gene containing a single Kr-binding site located near the 5' end of the 700-bp *rho* NEE (see diagram beneath the embryo). Neither enhancer is repressed, in that uniform staining is observed along the anteroposterior axis in both the presumptive mesoderm and neuroectoderm. The arrow shows the gap between the 2XPE and NEE expression domains. (B) Same as A except that the Kr-binding site was placed 75 bp upstream of the *lacZ* transcription start site. Both enhancers are repressed; mesoderm staining is nearly abolished in central regions containing high concentrations of Kr (2XPE enhancer), whereas the NEE lateral stripes are attenuated in the same domain.

Figure 6. The *sna* repressor can both quench and silence a modular *eve* promoter. Precellular transgenic embryos contain a modular promoter consisting of the 480-bp stripe 2 enhancer placed upstream of the 500-bp stripe 3 enhancer. A 160-bp spacer sequence separates the two enhancers. (A) *lacZ* staining pattern obtained with the fusion promoter containing four *sna* repressor sites inserted in the spacer sequence (see diagram beneath the embryo). These *sna* sites map far from activators in both enhancers (>150 bp). Consequently, neither enhancer is repressed, and both stripes are expressed uniformly in dorsal and ventral regions. Note that stripe 7 expression depends on regulatory sequences located in both enhancers, primarily the stripe 3 enhancer. In addition, the anterior head stripe (asterisk) is attributable to cryptic regulatory sequences in the P-transformation vector. (B) Same as A except that three *sna* repressor sites were inserted within the stripe 2 enhancer. This results in the ventral repression of stripe 2 (arrow). The neighboring stripe 3 enhancer is unaffected. (C) Same as A and B except that two *sna* sites were placed 55 bp from the *lacZ* transcription start site. Both stripes 2 and 3 are repressed in ventral regions (arrows), suggesting that *sna* functions as a dominant silencer when bound near the initiation site.

Figure 7. *sna* contains a discrete repression domain. Ventrolateral views of transgenic embryos expressing a modified *rho* NEE that lacks *sna* repressor sites and contains Gal4 recognition sequences (UAS; see summary diagrams beneath the embryos). (A) *lacZ* staining pattern obtained with the modified NEE in a normal embryo. A sharp staining pattern is observed in the ventral 20–22 cells, which encompasses the presumptive mesoderm and mesectoderm. (B) Same as A except that the modified NEE was crossed into an embryo that expresses a *sna*-Gal4 fusion protein in the ventral-most 14–16 cells. The NEE is repressed in regions containing the fusion protein.

sor sites in the *eve* stripe 2 enhancer overlap an activator site directly (Small et al. 1991, 1992). In particular, the *bcd* activator and *Kr* repressor recognize related binding sites (they share 7/10 matches), suggesting that *Kr* defines the posterior stripe 2 border via competitive binding to DNA (Driever et al. 1989; Stanojevic et al. 1989; Treisman and Desplan 1989). However, we have presented evidence that *Kr* need not function in this manner. It can repress the heterologous *rho* NEE even when completely uncoupled from *dl* activator sites. These results are consistent with our previous analysis of *sna* and raise the possibility that competition and quenching may not represent fundamentally different modes of repression.

The yeast $\alpha 2$ /Tup1 complex appears to function via direct repression of the transcription complex (Herschbach et al. 1994). Repression is observed over distances of several hundred base pairs, and genetic studies suggest that the Tup1 repressor interacts with one or more SRBs (suppressor of RNA polymerase B) in the RNA polymerase II (Pol II) holoenzyme complex (Herschbach et al. 1994; Barberis et al. 1995; Liao et al. 1995). It is unclear

Figure 8. Short-range repression is a flexible form of gene regulation. The diagram depicts a gene complex containing two enhancers placed between two divergently transcribed genes, *white* and *lacZ*. (A) Binding of a short-range repressor to enhancer 1. This enhancer is blocked from interacting with both the *white* and *lacZ* promoters (E1 off). However, the neighboring enhancer 2 is unaffected and activates both genes. In this configuration, the repressor permits enhancer autonomy (E2 is unaffected), but not promoter autonomy in that both genes are influenced by the repressor. (B) Binding of a short-range repressor to promoter-proximal regions of the *lacZ* gene. Both enhancers are blocked from interacting with *lacZ*, so there is no enhancer autonomy. However, only *lacZ* is repressed; *white* is unaffected, indicating that the two promoters are autonomous in their response to repressor. (C) Binding of a long-range silencer. In this case, the repressor acts over long distances to silence both *white* and *lacZ*. Both enhancers are blocked from interacting with both promoters. In principle, the silencer would exert the same effect on the gene complex regardless of location.

whether the $\alpha 2$ -Tup1 complex can also quench the neighboring Mcm1 activator or whether Tup1 must be linked closely to activators to contact the transcription complex.

Direct repression has been implicated in both *Drosophila* and mammalian tissue culture assays. *eve* has been shown to silence transcription, possibly through direct interactions with the TATA-binding protein (TBP) (Han and Manley 1993a; Um et al. 1995). A similar mechanism might apply to engrailed (Han and Manley 1993b), although there is also evidence that it can quench upstream activators when bound at a distance from the transcription start site (Jaynes and O'Farrell 1991). In vitro transcription assays suggest that the unliganded form of the thyroid hormone receptor might block transcription, over short distances, by directly contacting TBP or TFIIB (Fondell et al. 1993). Finally, other members of the nuclear receptor family, such as glucocorticoid receptor (GR), can work as repressors when bound >200 bp from the transcription start site (Pearce and Ya-

mamoto 1993) and in promoter-proximal regions (Drouin et al. 1993). Thus, previous studies on repressors raise a number of basic issues. Do they quench, directly repress the transcription complex, and work over short or long distances?

Our analyses of transcriptional repression in the early embryo suggest that there may be two basic forms of repression: short-range and long-range. We have analyzed the activities of six different repressors that are localized in precellular embryos: *sna*, *Kr*, *kni*, *giant* (*gt*), *dl*, and *hairy* (Gray et al. 1994; Paroush et al. 1994; Arnosti et al. 1996; Arnosti et al., in prep.). Four of the six embryos, *sna*, *Kr*, *kni*, and *gt*, appear to function over short distances, <100 bp, to either quench neighboring activators within a target enhancer or directly repress the transcription complex (this study; Arnosti et al., in prep.). In contrast, both *dl* (and associated corepressors) and *hairy* appear to work over long distances (≥ 1 kb) to silence the transcription complex (Ip et al. 1991; Jiang et al. 1993; Kirov et al. 1993; Lehming et al. 1994). For example, *dl*/corepressors can repress the ventral expression of the *eve* stripe 2 enhancer over a distance of ~ 5 kb (H. Cai and M. Levine, unpubl.). The *hairy* repressor can function in a dominant fashion and block multiple enhancers when bound ~ 1 kb upstream of the transcription start site (S. Barolo and M. Levine, unpubl.).

Mechanisms of short-range repression

Several different mechanisms of repression can be envisioned. The simplest view is that the short-range repressor directly inhibits upstream activators through protein-protein interactions. Perhaps *sna* and *Kr* repression domains block activator-promoter interactions. This direct mechanism of quenching may be compromised by the demonstration that both *sna* and *Kr* can silence the transcription complex when bound to promoter-proximal regions. However, it is conceivable that the repressors interact with upstream activators as they loop to the transcription complex. Alternatively, the repressors may recognize common motifs present in both upstream activators and the transcription complex (for review, see Goodrich and Tjian 1994).

Another potential mechanism involves the recruitment of one or more corepressors. As discussed above, the yeast $\alpha 2$ repressor recruits Tup1, which in turn inhibits the transcription complex (Hershbach et al. 1994). Moreover, *hairy* recruits groucho, which is related to Tup1 by virtue of WD40 repeats (Paroush et al. 1994). Perhaps *hairy* represses transcription through a groucho-promoter interaction similar to the situation described for $\alpha 2$ -Tup1. Recent studies suggest that the Tup1 repression complex is quite large, >1 million daltons, and contains multiple Tup1 and Ssn6 subunits (A.D. Johnson, pers. comm.). Short-range repressors, such as *Kr* and *sna*, might recruit smaller repression complexes that function only over short distances.

Tissue culture assays and in vitro binding experiments suggest that *Kr* may repress transcription by interacting with the TFIIE- β subunit of the transcription complex

(Sauer et al. 1995). Our results do not rule out such a mechanism; however, we note that the earlier study also suggests that Kr activates transcription through interactions with TFIIB. A model was presented whereby Kr monomers activate transcription, whereas dimers mediate repression. However, we have shown that a single Kr-binding site causes both the *rho* NEE and *twi* 2XPE enhancers to be repressed continuously in response to the Kr protein gradient in central regions of the embryo (e.g., Fig. 2). Activation is not observed at the margins of the pattern where there are diminishing levels of Kr protein.

Short-range repression in complex loci

The flexibility of short-range repression is particularly evident for complex genetic loci (summarized in Fig. 8). In the present study a complex locus was created by placing a modular promoter between two divergently transcribed reporter genes, *white* and *lacZ*. When either sna or Kr binds to a given enhancer (enhancer 1; see Fig. 8A), this enhancer is barred from interacting with both *white* and *lacZ*. However, the neighboring enhancer 2 is free to interact with both genes. In this case, local repression permits enhancers to function autonomously. In contrast, when sna or Kr repressor sites are placed near the *lacZ* transcription start site, both enhancer 1 and enhancer 2 are blocked (Fig. 8B). Despite this dominant repression of *lacZ*, both enhancers are unimpeded from interacting with the *white* gene. Thus, in this case, the short-range repressor permits promoter autonomy within a gene complex. This flexibility, enhancer autonomy or promoter autonomy, contrasts with the binding of long-range repressors within the complex, whereby both enhancers would be blocked on both promoters (Fig. 8C).

This form of repression might help explain localized patterns of homeotic gene expression within the Bithorax complex (BX-C). Repressors encoded by the gap genes *hunchback* (*hb*), *Kr*, and *knirps* (*kni*), have been shown to play a critical role in establishing the anterior boundaries of BX-C gene expression (Zhang et al. 1991; Qian et al. 1991; Busturia and Bienz 1993). For example, *Kr* binds within the *iab2* enhancer, which is located downstream of the *abdominal-A* (*abd-A*) gene (Shimell et al. 1994). *Kr* is required for keeping *abd-A* off, at least initially, in parasegment (PS) 6. It does not influence the expression of the neighboring *Ultrabithorax* (*Ubx*) gene, which is expressed in PS6, possibly because of the short-range nature of *Kr* repression.

Materials and methods

Plasmid constructions

The synthetic fusion promoter containing the 152-bp *rho* NEE and 480-bp *eve* stripe 2 enhancer (Fig. 1A) has been described previously (Gray et al. 1994). The 700-bp *rho* NEE (Ip et al. 1992b; Gray et al. 1994) was inserted into the *lacZ* 3' polylinker of the C4PLZ transformation vector (Wharton et al. 1993). sna sites were created in the 5' polylinker sequence by inserting a

synthetic 35-bp sequence, containing two sna sites (CAGCAAGGTG) separated by a 10-bp spacer, into either the *SphI* or the *KpnI* site. The spacer sequence separates the sna s2 and s3 sites in the native *rho* promoter (Ip et al. 1992b). The 2XPE *twi* enhancer was inserted between the *EcoRI* and *KpnI* sites of the C4PLZ vector; the 700-bp *rho* NEE (containing a synthetic Kr site) was cloned into the unique *BamHI* site. The *eve* stripe 2/stripe 3 fusion promoters (Fig. 6) were described previously (Small et al. 1993). Four sna sites were placed in the spacer between stripes 2 and 3 (Fig. 6A) by inserting two tandem copies of the 35-bp sequence, described above, into a synthetic *SphI* site located 150 bp 3' of the proximal *bcd* site in the stripe 2 enhancer. Two sna sites were placed 55 bp 5' of the *lacZ* transcription start (Fig. 6C) by inserting one copy of the 35-bp sequence into a synthetic *SphI* site located at the 3' end of the 500-bp stripe 3 enhancer. The sna sites in the stripe 2 enhancer (Fig. 6B) were created by site-directed mutagenesis (see below).

To construct the sna-Gal4 fusion sequence, an *XhoI*-*XbaI* fragment from the 2XPE *twi*-*lacZ* fusion gene (Jiang and Levine 1993) containing *twi* promoter sequences from -180 bp to +160 bp and the *lacZ* coding region was replaced by a truncated 1.6-kb *sna* promoter (Ip et al. 1992a), driving the *sna* cDNA through codon 244 fused to the 5' end of the coding region for Gal4 amino acids 2-93. A synthetic *NotI* site creates three alanine codons between *sna* amino acid 244 and Gal4 amino acid 2.

The UAS reporter (Fig. 7; modified from the 200-bp *rho* NEE described in Gray et al. 1994) was synthesized from a series of oligonucleotides and subcloned into the unique *NotI* and *BamHI* sites of the C4PLZ vector. Binding site identities were confirmed by sequence analysis. The dl 2, 3, and 4 sites and the *twi* t1 E box were unchanged. The *twi* t2 E box/sna s4 site and the E box/sna s3 site were changed to the *twi* t1 binding sequence (CATATG). The sna s2 site was replaced by a 15-bp dimeric Gal4-binding site (GGAGGACAGTCCTCC). Additional Gal4-binding sites were created at the 5' end of the modified NEE, and between the d3 and d4 activator sites. Gal4-binding sites are located (relative to the putative *rho* transcription start) as follows: -1735 to -1749 bp, -1842 to -1856 bp, and -1865 to -1879 bp.

P element-mediated germ-line transformation and whole-mount in situ hybridization

P-element transposons containing various *lacZ*/*white* reporter genes were introduced into the *Drosophila* germ line using standard methods (Spradling and Rubin 1982). Multiple independent transformed lines were examined for each fusion gene. Reporter gene expression was visualized by whole-mount in situ hybridization using digoxigenin-UTP-labeled *lacZ* or *white* antisense RNA probes (Tautz and Pfeifle 1989; Jiang et al. 1991).

Site-directed mutagenesis

The pBluescript SK+ plasmid and CJ236 strain of *Escherichia coli* were used to prepare uracil-containing single-stranded DNA templates. Mutagenesis was performed by annealing the templates with various mutagenic oligonucleotides. The in vitro-synthesized double-stranded DNA was transformed into the DH5 α strain of *E. coli*. The identities of the mutagenized templates were confirmed by sequence analysis.

Synthetic Kr sites (AAAACGGGTTAAGC) were created at the following positions in the *rho* NEE (all numbers refer to distance from the putative *rho* transcription start site): -2010 to -2023 bp, -1639 to -1652 bp, -2129 to -2142 bp, and -1533 to -1546 bp. Synthetic sna sites (CAGCAAGGTG)

were created in the *rho* NEE at –2012 to –2021 bp and –1649 to –1658 bp.

Synthetic *sna* sites were created in the *eve* stripe 2 enhancer at –1153 to –1162 bp, –1235 to –1244 bp, and –1386 to –1395 bp relative to the *eve* transcription start site.

Defective NEEs lacking native *sna*-binding sites were described by Ip et al. (1992b).

Acknowledgments

We thank Keith Maggert and Bob Zeller for help with computer imaging programs, and Liezelle de la Peña for technical assistance. We also thank Bill McGinnis and David Arnosti for critically reading the manuscript. This work was funded by grants from the National Institutes of Health (NIH) (GM34431 and GM46638).

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Alberga, A., J.L. Boulay, E. Kempe, C. Dennefeld, and M. Haclin. 1991. The *snail* gene required for mesoderm formation in *Drosophila* is expressed dynamically in derivatives of all three germ layers. *Development* **111**: 983–992.
- Arnosti, D.N., S. Barolo, M. Levine, and S. Small. 1996. The *eve* stripe 2 enhancer employs multiple modes of transcriptional synergy. *Development* **122**: 205–214.
- Barberis, A., J. Pearlberg, N. Simkovich, S. Farrell, P. Reinagel, C. Bamdad, G. Sigal, and M. Ptashne. 1995. Contact with a component of the polymerase II holoenzyme suffices for gene activation. *Cell* **81**: 359–368.
- Boulay, J.L., C. Dennefeld, and A. Alberga. 1987. The *Drosophila* developmental gene *snail* encodes a protein with nucleic acid binding fingers. *Nature* **330**: 395–398.
- Busturia, A. and M. Bienz. 1993. Silencers in *abdominal-B*, a homeotic *Drosophila* gene. *EMBO J.* **12**: 1415–1425.
- Cai, H. and M. Levine. 1995. Modulation of enhancer-promoter interactions by insulators in the *Drosophila* embryo. *Nature* **376**: 533–536.
- Chan, C.S., L. Rastelli, and V. Pirrotta. 1994. A *Polycomb* response element in the *Ubx* gene that determines an epigenetically inherited state of repression. *EMBO J.* **13**: 2553–2564.
- Chi, T., P. Lieberman, K. Ellwood, and M. Carey. 1995. A general mechanism for transcriptional synergy by eukaryotic activators. *Nature* **377**: 254–257.
- Cooper, J.P., S.Y. Roth, and R.T. Simpson. 1994. The global transcription regulators, SSN6 and TUP1, play distinct roles in the establishment of a repressive chromatin structure. *Genes & Dev.* **8**: 1400–1410.
- Driever, W., G. Thoma, and C. Nüsslein-Volhard. 1989. Determination of spatial domains of zygotic gene expression in the *Drosophila* embryo by the affinity of binding sites for the bicoid morphogen. *Nature* **340**: 363–367.
- Drouin, J., Y.L. Sun, M. Chamberland, V. Gauthier, A. De Lean, M. Nemer, and T.J. Schmidt. 1993. Novel glucocorticoid receptor complex with DNA element of the hormone-repressed POMC gene. *EMBO J.* **12**: 145–156.
- Fondell, J.D., A.L. Roy, and R.G. Roeder. 1993. Unliganded thyroid hormone receptor inhibits formation of a functional preinitiation complex: Implications for active repression. *Genes & Dev.* **7**: 1400–1410.
- Gaul, U. and H. Jackle. 1987. Pole region-dependent repression of the *Drosophila* gap gene *Krüppel* by maternal gene products. *Cell* **51**: 549–555.
- Genetta, T., D. Ruezinsky, and T. Kadesch. 1994. Displacement of an E-box-binding repressor by basic helix-loop-helix proteins: Implications for B-cell specificity of the immunoglobulin heavy-chain enhancer. *Mol. Cell. Biol.* **14**: 6153–6163.
- Gerwin, N., A. LaRosee, F. Sauer, H.P. Halbritter, M. Neumann, H. Jackle, and U. Nauber. 1994. Functional and conserved domains of the *Drosophila* transcription factor encoded by the segmentation gene *knirps*. *Mol. Cell. Biol.* **14**: 7899–7908.
- Goodrich, J.A. and R. Tjian. 1994. TBP-TAF complexes: Selectivity factors for eukaryotic transcription. *Curr. Opin. Cell Biol.* **6**: 403–409.
- Gray, S., P. Szymanski, and M. Levine. 1994. Short-range repression permits multiple enhancers to function autonomously within a complex promoter. *Genes & Dev.* **8**: 1829–1838.
- Han, K. and J.L. Manley. 1993a. Transcriptional repression by the *Drosophila* even-skipped protein: Definition of a minimal repression domain. *Genes & Dev.* **7**: 491–503.
- . 1993b. Functional domains of the *Drosophila* engrailed protein. *EMBO J.* **12**: 2723–2733.
- Herschbach, B.M., M.B. Arnaud, and A.D. Johnson. 1994. Transcriptional repression directed by the yeast $\alpha 2$ protein *in vitro*. *Nature* **370**: 309–311.
- Hoch, M., N. Gerwin, H. Taubert, and H. Jackle. 1992. Competition for overlapping sites in the regulatory region of the *Drosophila* gene *Krüppel*. *Science* **256**: 94–97.
- Huang, J.D., D.H. Schwyter, J.M. Shirokawa, and A.J. Courey. 1993. The interplay between multiple enhancer and silencer elements defines the pattern of *decapentaplegic* expression. *Genes & Dev.* **7**: 694–704.
- Ip, Y.T., R. Kraut, M. Levine, and C.A. Rushlow. 1991. The dorsal morphogen is a sequence-specific DNA-binding protein that interacts with a long-range repression element in *Drosophila*. *Cell* **64**: 439–446.
- Ip, Y.T., R.E. Park, D. Kosman, K. Yazdanbakhsh, and M. Levine. 1992a. Dorsal-twist interactions establish *snail* expression in the presumptive mesoderm of the *Drosophila* embryo. *Genes & Dev.* **6**: 1518–1530.
- Ip, Y.T., R.E. Park, D. Kosman, E. Bier, and M. Levine. 1992b. The dorsal gradient morphogen regulates stripes of *rhomboid* expression in the presumptive neuroectoderm of the *Drosophila* embryo. *Genes & Dev.* **6**: 1728–1739.
- Ip, Y.T., K. Maggert, and M. Levine. 1994. Uncoupling gastrulation and mesoderm differentiation in the *Drosophila* embryo. *EMBO J.* **13**: 5826–5834.
- Jaynes, J.B. and P.H. O’Farrell. 1991. Active repression of transcription by the engrailed homeodomain protein. *EMBO J.* **10**: 1427–1433.
- Jiang, J. and M. Levine. 1993. Binding affinities and cooperative interactions with bHLH activators delimit threshold responses to the dorsal gradient morphogen. *Cell* **5**: 741–752.
- Jiang, J., H. Cai, Q. Zhou, and M. Levine. 1993. Conversion of a dorsal-dependent silencer into an enhancer: evidence for dorsal corepressors. *EMBO J.* **12**: 3201–3209.
- Jiang, J., D. Kosman, Y.T. Ip, and M. Levine. 1991. The dorsal morphogen gradient regulates the mesoderm determinant *twist* in early *Drosophila* embryos. *Genes & Dev.* **5**: 1881–1891.
- Johnson, A.D. 1995. The price of repression. *Cell* **81**: 655–658.
- Keleher, C.A., M.J. Redd, J. Schultz, M. Carlson, and A.D. Johnson. 1992. Ssn6-Tup1 is a general repressor of transcription in yeast. *Cell* **68**: 709–719.
- Kirov, N., L. Zhelnin, J. Shah, and C.A. Rushlow. 1993. Con-

- version of a silencer into an enhancer: Evidence for a co-repressor in dorsal-mediated repression in *Drosophila*. *EMBO J.* **12**: 3193–3199.
- Kosman, D., Y.T. Ip, M. Levine, and K. Arora. 1991. Establishment of the mesoderm-neuroectoderm boundary in the *Drosophila* embryo. *Science* **254**: 118–122.
- Lehming, N., D. Thanos, J.M. Brickman, J. Ma, T. Maniatis, and M. Ptashne. 1994. An HMG-like protein that can switch a transcriptional activator to a repressor. *Nature* **371**: 175–179.
- Leptin, M. 1991. twist and snail as positive and negative regulators during *Drosophila* mesoderm development. *Genes & Dev.* **5**: 1568–1576.
- Levine, M. and J.L. Manley. 1989. Transcriptional repression of eukaryotic promoters. *Cell* **59**: 405–408.
- Liao, S.M., J. Zhang, D.A. Jeffery, A.J. Koleske, C.M. Thompson, D.M. Chao, M. Viljoen, H.J. van Vuuren, and R.A. Young. 1995. A kinase-cyclin pair in the RNA polymerase II holoenzyme. *Nature* **374**: 193–196.
- Licht, J.D., M. Ro, M.A. English, M. Grossel, and U. Hansen. 1993. Selective repression of transcriptional activators at a distance by the *Drosophila* Kruppel protein. *Proc. Natl. Acad. Sci.* **90**: 11361–11365.
- Licht, J.D., W. Hanna-Rose, J.C. Reddy, M.A. English, M. Ro, M. Grossel, R. Shaknovich, and U. Hansen. 1994. Mapping and mutagenesis of the amino-terminal transcription repression domain of the *Drosophila* Kruppel protein. *Mol. Cell. Biol.* **14**: 4057–4066.
- Mauhin, V., Y. Lutz, C. Dennefeld, and A. Alberga. 1993. Definition of the DNA-binding site repertoire for the *Drosophila* transcription factor snail. *Nucleic Acids Res.* **21**: 3951–3957.
- Pan, D., J.D. Huang, and A.J. Courey. 1991. Functional analysis of the *Drosophila* twist promoter reveals a dorsal-binding ventral activator region. *Genes & Dev.* **5**: 1892–1901.
- Pankratz, M.J. and H. Jäckle. 1990. Making stripes in the *Drosophila* embryo. *Trends Genet.* **6**: 287–292.
- Paro, R. 1993. Mechanisms of heritable gene repression during development of *Drosophila*. *Curr. Opin. Cell Biol.* **5**: 999–1005.
- Paroush, Z., R.L. Finley Jr., T. Kidd, S.M. Wainwright, P.W. Ingham, R. Brent, and D. Ish-Horowicz. 1994. groucho is required for *Drosophila* neurogenesis, segmentation, and sex determination and interacts directly with hairy-related bHLH proteins. *Cell* **79**: 805–815.
- Pearce, D. and K.R. Yamamoto. 1993. Mineralocorticoid and glucocorticoid receptor activities distinguished by nonreceptor factors at a composite response element. *Science* **259**: 1161–1165.
- Qian, S., M. Capovilla, and V. Pirrotta. 1991. The *bx* region enhancer, a distant cis-control element of the *Drosophila* *Ubx* gene and its regulation by *hunchback* and other segmentation genes. *EMBO J.* **10**: 1415–1425.
- Sauer, F., J.D. Fondell, Y. Ohkuma, R.G. Roeder, and H. Jackle. 1995. Control of transcription by Kruppel through interactions with TFIIB and TFIIE β . *Nature* **375**: 162–164.
- Sawada, S., J.D. Scarborough, N. Killeen, and D.R. Littman. 1994. A lineage-specific transcriptional silencer regulates CD4 gene expression during T lymphocyte development. *Cell* **77**: 917–929.
- Shimell, M.J., J. Simon, W. Bender, and M.B. O'Connor. 1994. Enhancer point mutation results in a homeotic transformation in *Drosophila*. *Science* **264**: 968–971.
- Simpson-Brose, M., J. Treisman, and C. Desplan. 1994. Synergy between the *hunchback* and *bicoid* morphogens is required for anterior patterning in *Drosophila*. *Cell* **78**: 855–865.
- Siu, G., A.L. Wurster, D.D. Duncan, T.M. Soliman, and S.M. Hedrick. 1994. A transcriptional silencer controls the developmental expression of the CD4 gene. *EMBO J.* **13**: 3570–3579.
- Small, S. and M. Levine. 1991. The initiation of pair-rule stripes in the *Drosophila* blastoderm. *Curr. Opin. Genet. Dev.* **1**: 255–260.
- Small, S., R. Kraut, T. Hoey, R. Warrior, and M. Levine. 1991. Transcriptional regulation of a pair-rule stripe in *Drosophila*. *Genes & Dev.* **5**: 827–839.
- Small, S., A. Blair, and M. Levine. 1992. Regulation of *even-skipped* stripe 2 in the *Drosophila* embryo. *EMBO J.* **11**: 4047–4057.
- Small, S., D.N. Arnosti, and M. Levine. 1993. Spacing ensures autonomous expression of different stripe enhancers in the *even-skipped* promoter. *Development* **119**: 767–772.
- Small, S., A. Blair, and M. Levine. 1996. Regulation of two pair-rule stripes by a single enhancer in the *Drosophila* embryo. *Dev. Biol.* (in press).
- Spradling, A.C. and G.M. Rubin. 1982. Transposition of cloned P elements into *Drosophila* germline chromosome. *Science* **218**: 341–347.
- Stanojevic, D., T. Hoey, and M. Levine. 1989. Sequence-specific DNA-binding activities of the gap proteins encoded by *hunchback* and *Krüppel* in *Drosophila*. *Nature* **341**: 331–335.
- Stanojevic, D., S. Small, and M. Levine. 1991. Regulation of a segmentation stripe by overlapping activators and repressors in the *Drosophila* embryo. *Science* **254**: 1385–1387.
- Stromstedt, P.E., L. Poellinger, J.A. Gustafsson, and J. Carlstedt-Duke. 1991. The glucocorticoid receptor binds to a sequence overlapping the TATA box of the human osteocalcin promoter: A potential mechanism for negative regulation. *Mol. Cell. Biol.* **11**: 3379–3383.
- Studer, M., H. Popperl, H. Marshall, A. Kuroiwa, and R. Krumlauf. 1994. Role of a conserved retinoic acid response element in rhombomere restriction of *Hoxb-1*. *Science* **265**: 1728–1732.
- Tautz, D. and C. Pfeifle. 1989. A nonradioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals a translational control of the segmentation gene *hunchback*. *Chromosoma* **98**: 81–85.
- Thisse, C., F. Perrin-Schmitt, C. Stoetzel, and B. Thisse. 1991. Sequence-specific transactivation of the *Drosophila* *twist* gene by the *dorsal* gene product. *Cell* **65**: 1191–1201.
- Treisman, J. and C. Desplan. 1989. The products of the *Drosophila* gap genes *hunchback* and *Krüppel* bind to the *hunchback* promoters. *Nature* **341**: 335–357.
- Um, M., C. Li, and J.L. Manley. 1995. The transcriptional repressor *even-skipped* interacts directly with TATA-binding protein. *Mol. Cell. Biol.* **15**: 5007–5016.
- Vershon, A.K. and A.D. Johnson. 1993. A short, disordered protein region mediates interactions between the homeodomain of the yeast $\alpha 2$ protein and the MCM1 protein. *Cell* **72**: 105–112.
- Wharton, K.A. Jr. and S.T. Crews. 1993. CNS midline enhancers of the *Drosophila* *slit* and *Toll* genes. *Mech. Dev.* **40**: 141–154.
- Zhang, C.C., J. Muller, M. Hoch, H. Jackle, and M. Bienz. 1991. Target sequences for *hunchback* in a control region conferring *Ultrabithorax* expression boundaries. *Development* **113**: 1171–1179.
- Zuo, P., D. Stanojevic, J. Colgan, K. Han, M. Levine, and J. L. Manley. 1991. Activation and repression of transcription by the gap proteins *hunchback* and *Krüppel* in cultured *Drosophila* cells. *Genes & Dev.* **5**: 254–264.

Short-range transcriptional repressors mediate both quenching and direct repression within complex loci in *Drosophila*.

S Gray and M Levine

Genes Dev. 1996, **10**:

Access the most recent version at doi:[10.1101/gad.10.6.700](https://doi.org/10.1101/gad.10.6.700)

References

This article cites 69 articles, 29 of which can be accessed free at:
<http://genesdev.cshlp.org/content/10/6/700.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).