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Genetics

Functional redundancy: The respective roles of the two sloppy paired genes in *Drosophila* segmentation

(engrailed/wingless/segment polarity)

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ABSTRACT **The sloppy paired (*slp*) locus consists of two genes, *slp1* and *slp2*, both of which encode proteins containing a forkhead domain (a DNA-binding motif). Previous work has shown that a severe segmentation phenotype is obtained only when both *slp* genes are deleted. Here we examine the functional redundancy of the locus in more detail. The phenotypes of embryos containing various combinations of functional *slp* genes suggest that for early *slp* function, until gastrulation, only *slp1* is required. At later times, there is still a greater requirement for *slp1*, but in many respects the two *slp* genes are completely redundant. Both *slp* genes produce similar phenotypes when ubiquitously expressed via a heat shock promoter. We propose that the slp proteins are biochemically equivalent and that the greater requirement for slp1 in some functions can be explained in large part by its earlier expression.**

Much of our knowledge concerning pattern formation in the *Drosophila* embryo originates from exhaustive genetic screens for mutations that specifically affect segmentation (1–4). Such screens can only identify genes that, when mutated in an otherwise wild-type background, display a recognizable phenotype. Gene duplication during evolution raises the question of whether functional redundancy between structurally related genes would preclude their isolation in such screens. There are several well-established cases of this in yeast—i.e., where two proteins are believed to have identical biochemical functions so that a phenotype is only observed when both are mutated (5–7). At the present time, there are few reports addressing such redundancy in *Drosophila*: the best studied example is that of the BarH1 and BarH2 genes of the Bar (*B*) locus (8).

Molecular analysis of several of the segmentation genes identified in the aforementioned screens revealed the existence of a closely linked, structurally related gene that is expressed in a similar, sometimes identical pattern. The available evidence suggests that these genes arose by tandem duplication and eventual translocation to a nearby chromosomal site. Some of these gene pairs include engrailed (*en*) and invected (9, 10), knirps (*kni*) and knirps-related (*knrl*; refs. 11 and 12), and gooseberry and gooseberry-neuro (also known as *gsb* proximal and *gsb* distal; refs. 13 and 14). In all these cases, only the first gene mentioned of the pair is believed to contribute to segmentation (10, 15, 16), although an intronless *knrl* gene can rescue the *kni* mutant phenotype when placed under the control of the *kni* promoter (15).

The sloppy paired (*slp*) locus also contains two related genes, *slp1* and *slp2* (17), identified in an enhancer detection screen (18–20), which allows the identification of functionally redundant genes. The original *slp* mutants, isolated in a saturating ethyl methanesulfonate mutagenesis screen (2), caused a modest pair-rule phenotype and are hypomorphic alleles of *slp1* (17). No mutants corresponding to *slp2* were found in this screen, presumably because these mutants had no phenotype or one that was too subtle to detect. In contrast, small deletions removing both *slp* genes result in a severe pair-rule/segment polarity phenotype (17, 21). Both slp proteins contain a forkhead domain, a DNA-binding motif (22, 23).

This report examines the redundancy of the *slp* locus in more detail, using different combinations of *slp* mutations, as well as examining the effect of ubiquitous expression of both genes. The data suggest that the two *slp* genes can perform the same tasks, but due to its earlier expression, only *slp1* is required for the early functions of the locus.

MATERIALS AND METHODS

Fly Stocks. slp^{7148}, $Df(2L)ed^{SZ1}$, the enhancer trap line CyO P[lArB]A208.1M2 and its derivatives CyOΔ46G and Δ34B have been described (17). The creation of the *slp* heat shock (P[HS-slp]) lines is described elsewhere (21). The *slp1* rescue construct P[slp1] contains the *slp1* coding region flanked by ≈14 kb upstream of the putative transcription start site and 2 kb downstream of the poly(A) site (the gene contains no introns; ref. 17) cloned into the *P*-element vector C20.1 (24). A detailed outline of the cloning is available upon request. Germ-line transformants were obtained by microinjection (25) using pπ25.7wc as a source of transposase (26).

Cuticle Preparations. Cuticles were prepared as described (2) with some modifications (21).

Analysis of Expression Patterns. Embryo antibody stainings were performed essentially as described by Frasch *et al.* (27). Rabbit anti-fushi tarazu (*ftz*) antisera (28) was a gift from H. Krause (University of Toronto) and alkaline phosphatase-conjugated swine anti-rabbit antibody was from Dakopatts (Glostrup, Denmark). Both antibodies were used at a 1:200 dilution. *In situ* hybridizations using digoxygenin-labeled probes were done according to Tautz and Pfeifle (29) with some modifications (detailed protocol available upon request). DNA templates were cDNAs from the following sources: *en*, pF7036 (30); *ftz*, pGEMf1 (28); wingless (*wg*), pCV (31) was from M. van den Heuvel and R. Nusse (Stanford University); hedgehog (*hh*), chh46 (32) was from S. Tabata and T. Kornberg and even-skipped (*eve*), pGEM1-eve was from S. Small (New York University).

RESULTS

Cuticle Phenotypes of Different *slp* Alleles. The cuticle phenotypes of Δ34B, Δ46G, and Δ46G/Δ34B embryos have

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Abbreviation: PS, parasegment.

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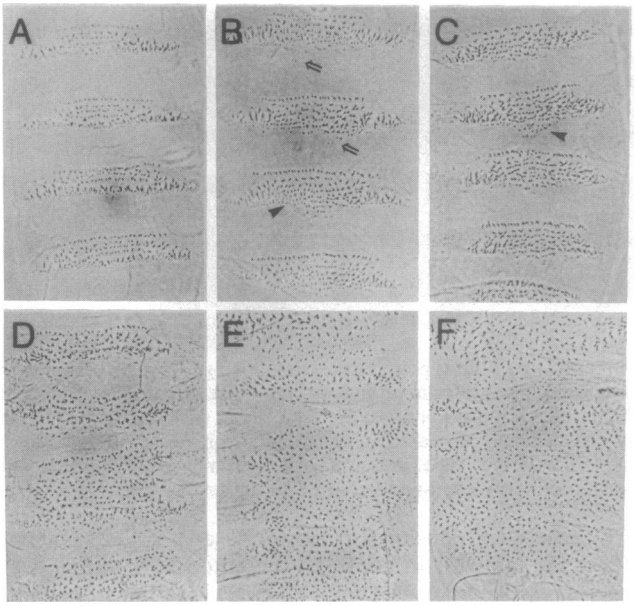

FIG. 1. Phase-contrast micrographs of the ventral cuticles of various *slp* mutant combinations. (*A*) Abdominal segments three through six (A3–A6) of an embryo homozygous for enhancer trap insert P[lArB]208.1M2 from which deletion mutants Δ46G and Δ34B were derived. The pattern is essentially like that in wild type where each segment contains six rows of denticles, one anterior and five posterior of the segment border. The posterior half of the segment (except for the first denticle row) is devoid of denticles (naked cuticle). (*B*) A3–A6 of a Δ34B embryo (deleting *slp1* and *slp2*) containing two copies of a *P* element containing genomic *slp1* sequences (P[slp1]). Note that the denticle belts appear slightly wider, with A5 possessing an entire extra row of denticles posterior to row six (arrowhead). A few denticles posterior to this seventh row are also found in each segment (open arrows). (*C*) Δ34B embryo containing one copy of P[slp1]. The phenotype is more severe than that in *B*, with A4 displaying a small cluster of denticles where only naked cuticle is normally found (arrowhead). (*D*) A3–A7 of a Δ46G embryo (deleting *slp1* and leaving *slp2* intact). Denticle belts are fused in pair-rule fashion (this particular embryo shows a slight fusion of A3 and A4, but A5 and A6 are completely merged. Note there is still substantial amounts of naked cuticle. (*E*) A3–A8 of a Δ46G/Δ34B embryo. Pair-rule fusions are more severe, and most naked cuticle is gone. (*F*) A1–A8 of a Δ34B embryo. Embryos are much smaller than wild type, and virtually all the naked cuticle is now gone.

been reported (17), and phase-constrast micrographs of ventral abdomens of these mutants are shown in Fig. 1. Δ34B is a deficiency deleting both *slp* genes (17), and homozygous Δ34B embryos exhibit fusions of segments and a lack of naked cuticle (Fig. 1*F*). Δ46G is a deficiency removing only *slp1* and has no detectable effect on *slp2* expression. Existing ethyl methanesulfonate-induced *slp* alleles, such as slp^{7L48} (2) fail to complement Δ46G (17). Δ46G embryos have partial fusion of segments in a pair-rule fashion, but naked cuticle is still present (Fig. 1*D*). Δ46G/Δ34B transheterozygotes (i.e., null for *slp1* and hemizygous for *slp2*), have an intermediate phenotype (Fig. 1*E*), suggesting that both *slp* genes function in the same pathway (17).

Both the Δ46G and Δ34B deficiencies are on the balancer chromosome CyO (17). Embryos homozygous for CyO P[lArB]A208.1M2, the parental chromosome of Δ46G and Δ34B, failed to hatch but have no defect in segmentation (Fig. 1*A*). Moreover, when $Df(2L)ed^{SZ1}$, a non-CyO chromosome containing a large deficiency removing both *slp* genes, is combined with Δ46G or Δ34B, the transheterozygous embryos have identical cuticle phenotypes as Δ46G/Δ34B and Δ34B/Δ34B mutants, respectively (data not shown). Therefore, the CyO chromosomal background does not contribute to the segmentation defects described here.

No mutations mapping to *slp2* are available, and efforts to create *slp2*-specific deficiencies by imprecise excision of a nearby *P*-element have been unsuccessful (R. Kurth Pearson, K.M.C., and W.J.G., unpublished results). The *slp2* mutant phenotype was therefore examined in Δ34B homozygous embryos containing one or two copies of a *P*-element *slp1* rescue construct (P[slp1]). One copy of P[slp1] can rescue $\Delta 46G/slp^{7L48}$ flies to adulthood (data not shown) and dramatically rescues the Δ34B segmentation defect, though some of the naked cuticle is still replaced by extra denticles (Fig. 1*C*). Two copies of P[slp1] provide more rescue, but extra denticles are still present (Fig. 1*B*), indicating that embryos lacking *slp2* have a weak segment polarity phenotype. Due to the embryonic lethality of CyO homozygotes, we could not determine whether P[slp1];Δ34B embryos survive past embryogenesis.

Segmentation Gene Expression in *slp* Mutants. One of the major targets of *slp* action is the *wg* gene (21). In Δ34B mutants, the *wg* stripes in every even-numbered parasegment (PS) fade during germ-band extension (Fig. 2*E*), and the remaining stripes disappear when the germ band is fully extended (Fig. 2*F*). *wg* is needed for the differentiation of naked cuticle (1, 33–35), and the lack of naked cuticle in Δ34B is most likely due to the premature fading of *wg* transcripts. In Δ46G embryos, *wg* expression partially fades in each even-numbered PS (Fig. 2*A*), but all the *wg* stripes are still visible (the odd-numbered ones at wild-type levels) at a time when they are completely gone in Δ34B embryos (Fig. 2*B*). This demonstrates that *slp2* alone can maintain *wg* expression

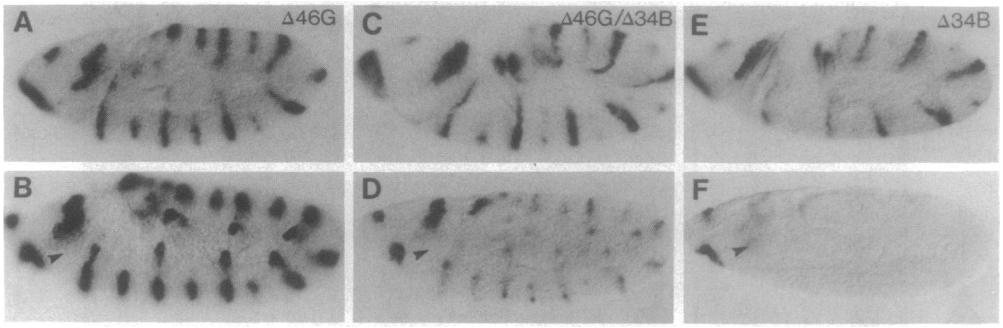

FIG. 2. Expression of *wg* in *slp* mutants. In Δ46G embryos, the *wg* stripes in every even-numbered PS fade during germ-band extension to the point shown in *A* (late stage 9). At late stage 10 (*B*), the odd-numbered PS stripes are still expressed at wild-type levels, and the even-numbered ones, while weak (note the lateral domains are absent), still persist. In Δ46G/Δ34B embryos, the even-numbered stripes are very faint at stage 9 (*C*), and the remaining stripes fade during stage 10 (*D*). In Δ34B embryos, the even-numbered stripes are gone by midstage 9 (*E*), and all *wg* expression in the trunk is absent by early stage 10 (*F*). Arrowheads in *B*, *C*, and *F* point to where the antennal segment staining is found in wild type; it is absent in all three panels.

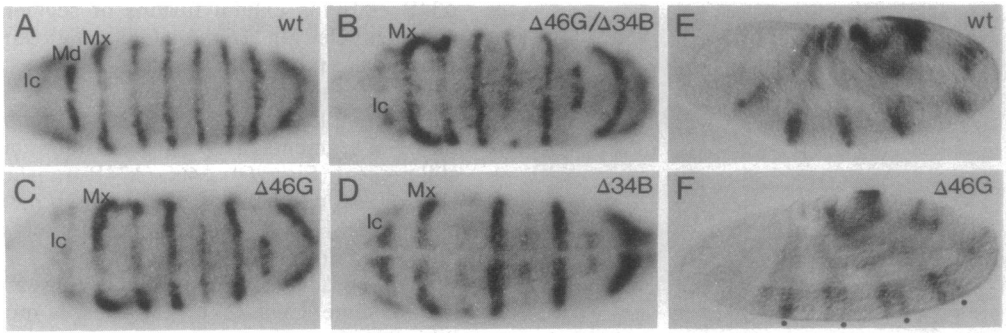

FIG. 3. Expression of *en* mRNA (*A–D*) and ftz protein (*E* and *F*) in *slp* mutants. (*A*) Wild-type *en* expression at stage 10. Expression in the intercalary (Ic), mandibular (Md), and maxillary (Mx) segment primordia are labeled (the intercalary domains in *A–C* are out of focus but appear similar). In Δ46G/Δ34B (*B*) and Δ46G (*C*) embryos, the *en* stripes in each odd-numbered PS are greatly reduced. In Δ34B embryos (*D*), the odd-numbered PS stripes are almost gone, and every stripe is significantly broader than normal. The mandibular domain is missing and the maxillary stripe is wider than wild type in all three *slp* mutant combinations. (*E*) Wild-type *ftz* expression. In Δ46G embryos (*F*) and stronger *slp* mutants (data not shown), there are extra *ftz* stripes in the posterior portion of every even-numbered PS (indicated by dots).

to a significant extent. As with the cuticle defect, Δ46G/Δ34B transheterozygotes show an intermediate phenotype between the two mutants (Fig. 2 *C* and *D*). No detectable defect in *wg* expression in Δ34B; P[slp1]/+ embryos was observed (data not shown). The cuticular phenotype of these embryos is very similar to that of embryos homozygous for a temperature-sensitive *wg* mutation that were switched to the restrictive temperature during the later half of germ-band shortening (34). Perhaps embryos lacking *slp2* have a reduction in the level of *wg* transcripts that is impossible to detect with the whole-mount staining techniques that were employed. In summary, both *slp* genes are required for proper maintenance of *wg* expression, but *slp1* plays a more prominent role.

In addition to its function as an activator of *wg* expression, *slp* is also involved in the repression of several genes. In Δ34B embryos, the expression domains of *eve*, *en*, and *hh* expand anteriorly, into the cells where the *slp* genes are normally expressed. In addition, an extra stripe of *ftz* expression is seen in the posterior part of each even-numbered PS in Δ34B embryos, again in the domain of normal *slp* expression (21). The expression of *en* in various *slp* mutants is shown in Fig. 3. During germ-band extension, *wg* is required for the maintenance of *en* expression (34, 36), so the decay of the odd-numbered PS *en* stripes in all three *slp* mutants shown is due to the disappearance of the adjacent even-numbered PS *wg* stripes (21). In contrast, the widening of the *en* stripes is only seen when both *slp* genes are removed (compare Fig. 3 *A–C* with 3*D*). Identical results were obtained for *hh* and *eve*; i.e., anterior expansion was also only seen in Δ34B embryos (data not shown). In contrast, the ectopic *ftz* stripes were seen in Δ46G embryos (Fig. 3*F*) as well as the stronger *slp* mutants, but not in Δ34B;P[slp1]/+ embryos (data not shown), indicating that *slp2* has no role in *ftz* repression.

Like *ftz* repression, *slp2* has no detectable role in the defects seen in the pregnathal and gnathal segment primordia in *slp* mutants (refs. 17 and 21; unpublished results), which occur in Δ46G and Δ34B mutants but not in Δ34B; P[slp1]/+ embryos. These include the lack of *en* expression in the mandibular segment, widening of the *en* maxillary stripe (Fig. 3 *A–D* and data not shown), and a lack of *wg* transcripts in the antennal segment (Fig. 2 and data not shown).

Effect of Ubitquitous Expression of *slp1* or *slp2*. To examine the effects of ectopic *slp* expression, both *slp* coding sequences were placed under the control of the *hsp70* heat shock promoter, and transgenic flies (P[HS-slp]) were created (21). In all experiments described below, no significant differences between the phenotypes generated by P[HS-slp1], P[HS-slp2], or P[HS-slp1]; P[HS-slp2] embryos were observed. There were quantitative differences in terms of the strength of phenotypes between individual lines of each construct, but the overlap was so considerable that, at least with the assays employed, P[HS-slp1] and P[HS-slp2] embryos were roughly equivalent. The three lines examined in detail can be arranged by the strength of their induced phenotypes P[HS-slp2] > P[HS-slp1C] > P[HS-slp1A].

The cuticle phenotypes generated by P[HS-slp] embryos after heat shock fall into four classes. Fig. 4*A* shows a cuticle

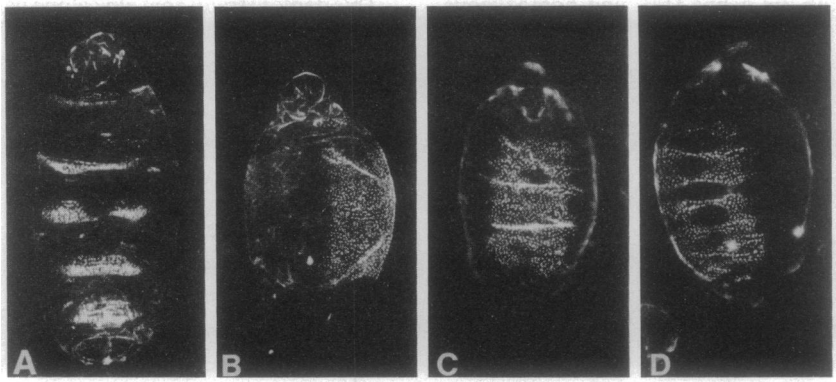

FIG. 4. Phenotypes observed after ubiquitous expression of slp proteins. (*A*) P[HS-slp1A] embryo that had received two 5-min heat shocks (at 37°C), the first at syncitial blastoderm and the second 55 min later. The phenotype is similar to that of a weak *eve* mutant; i.e., the denticle belts of the second, fourth, sixth, and eighth abdominal segments are missing. (*B*) P[HS-slp2] embryo treated as in *A*. The phenotype resembles that found in null *eve* alleles. A similar phenotype (though not as penetrant) was found in P[HS-slp1C] embryos (data not shown). (*C* and *D*) P[HS-slp2] embryos that received the first of two heat shocks during early germ-band extension. Approximately half the embryos had a phenotype like that in *C*, which resembles a strong *en* or *hh* mutant. The other half resembled the cuticle in *D*, which is similar to some *en* mutants.

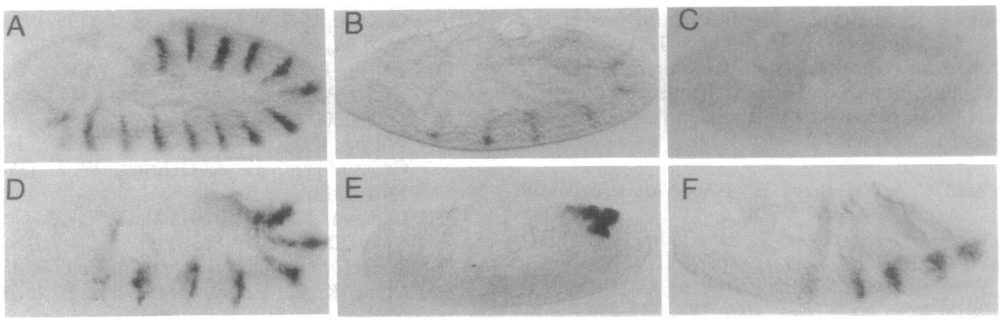

FIG. 5. Expression of *en* (*A–C*), *eve* (*D* and *E*), and *ftz* (*F*) transcripts after ubiquitous expression of *slp1* or *slp2*. Control embryos containing the heat shock vector alone (*A* and *D*) were fixed 40 min after two 10-min heat shocks (separated by 50 min). The other P[HS-slp1] or P[HS-slp2] embryos were treated the same as controls except the heat shocks were for only 5 min each. (*A*) The expression of *en* is normal in control embryos. (*B*) P[HS-slp1A] embryo. Note only the stripes in each even-numbered PS remain, and they are faint. (*C*) P[HS-slp2] embryo. *en* expression has been completely abolished. (*D*) The seven eve stripes are still strongly expressed in control embryos. (*E*) P[HS-slp1A] embryo showing lack of eve staining (except for the seventh stripe and anal pad primodia). (*F*) P[HS-slp1C] embryo. *ftz* transcription is repressed to variable levels but, as shown here, the anterior stripes are more sensitive to *slp* repression.

commonly obtained in weaker lines (e.g., P[HS-slp1A]) that received their first heat shock at syncitial blastoderm. Stronger lines induced at this time result in cuticles like that shown in Fig. 4*B*. These phenotypes are quite similar to hypomorphic and amorphic *eve* mutants (37, 38). When P[HS-slp2] embryos are first heat shocked at early germ-band extension, many cuticles develop like that shown in Fig. 4*C*. The remainder of the P[HS-slp2] and the majority of P[HS-slp1C] embryos look similar to the one shown in Fig. 4*D*. These cuticles have many similarities with those of *hh* (3, 39) or *en* mutants (1, 40).

Consistent with the cuticle phenotypes, P[HS-slp] was found to repress the expression of *en*, *hh*, *eve*, and *ftz* (Fig. 5 and data not shown). The order in terms of susceptibility to repression was *eve* $\geq$ *en* = *hh* > *ftz*. The inhibition of the first three was very consistent, but *ftz* repression showed a wide variation, though the first two stripes were almost always repressed (Fig. 5*F*).

In a patched (*ptc*) mutant background, ubiquitous expression of *slp1* caused a near ubiquitous expression of *wg* (21). Overexpression of *slp2* in *ptc* mutants was found to have a similar effect on *wg* expression as *slp1* (data not shown).

DISCUSSION

Previous work (17) and this study demonstrate that the *slp* genes act with partial redundancy in embryonic segmentation. We can group the phenotypes seen in *slp* mutants into three classes based on the redundancy observed. (*i*) *slp1* is solely responsible for the misexpression of *en* and *wg* in the pregnathal and gnathal segments and for *ftz* derepression. (*ii*) Normal maintenance of *wg* transcripts is dependent on the presence of both *slp1* and *slp2*, though the P[slp1] rescue experiment indicates a greater requirement for the *slp1* locus. (*iii*) Repression of *eve*, *en*, and *hh* expression can be achieved by either *slp* gene. In constrast to this partial redundancy in the loss of function mutant analysis, *slp1* and *slp2* appear to be completely redundant in the heat shock experiments, suggesting that both slp proteins have similar biochemical capabilities, at least when overexpressed.

An explanation for the greater requirement for *slp1* in some functions may lie in differences in expression between the *slp* genes. The *slp* transcripts are expressed in spatially identical patterns throughout most of embryogenesis (17) as are the proteins (21). However, there are spatial and temporal differences in early embryos. *slp1* is expressed in a much broader region of the head at syncitial blastoderm than *slp2* (17), which could explain the lone requirement for *slp1* in the regulation of *en* and *wg* in this area. The ectopic expression of *ftz* is the earliest abnormality seen in the trunk of *slp* mutants, beginning during gastrulation. At this time, the *slp1* striped pattern is well established, but *slp2* expression is still evolving (17), hence the lack of a requirement for *slp2* in *ftz* derepression.

It is difficult to explain the greater role of *slp1* in the maintenance of *wg* expression because this regulation occurs at a time when the *slp2* expression pattern is fully established. Perhaps the absolute levels of slp2 protein are significantly lower than those of slp1, so that the role of *slp2* in maintaining *wg* expression is only dramatically demonstrated in a *slp1* mutant background—i.e., *wg* transcripts fade much sooner in Δ34B (lacking both *slp* genes) than in Δ46G embryos (lacking only *slp1*). Repression of *en*, *hh*, and *eve* expression may only require a small amount of *slp* activity, so even the lower level of slp2 protein is sufficient for proper repression in the absence of slp1. This model can best be tested by placing the *slp2* coding region under control of the *slp1* promoter. If the slp1 and slp2 proteins are functionally equivalent, as our data suggest, then the chimeric construct should rescue the slp$^-$ phenotype as well as P[slp1]. A similar type of experiment has been performed to demonstrate that the knrl protein is functionally equivalent to kni protein. When placed under control of the *kni* promoter, *knrl* (provided its large intron is removed) can completely rescue *kni* mutants (15).

It is perhaps surprising that the two slp proteins appear to be biochemically equivalent, since, outside of the forkhead domain (a 107-amino-acid domain where they are 76% identical), they are highly diverged, except for a few short regions of homology (17). These short stretches of homology are also found in other *Drosophila* and mammalian members of the forkhead gene family (17). The importance of these sequences can now be tested, in either the *slp* rescue assay or the heat shock experiments described in this report.

In conclusion, the *slp* locus serves as a cautionary tale for researchers wishing to elucidate complex genetic pathways. Until recently, *slp* received little attention in models of segmentation, because of the modest phenotype of *slp1* mutants. The pivotal role of the locus was only recognized after deficiencies removing both genes were created (17, 21). As outlined in the Introduction, several of the other segmentation loci also have a nearby, structurally related partner, though these genes don't seem to contribute to the segmentation phenotype (9–16). This raises the question: are there other loci acting in this hierarchy that have been missed in genetic screens because their redundancy was more complete than that of *slp* or the other known gene pairs?

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1. Nüsslein-Volhard, C. & Wieschaus, E. (1980) *Nature (London)* **287**, 795–801.
2. Nüsslein-Volhard, C., Wieschaus, E. & Kluding, H. (1984) *Wilhelm Roux Arch. Dev. Biol.* **193**, 267–282.
3. Jürgens, G., Wieschaus, E., Nüsslein-Volhard, C. & Kluding, H. (1984) *Wilhelm Roux Arch. Dev. Biol.* **193**, 283–295.
4. Wieschaus, E., Nüsslein-Volhard, C. & Jürgens, G. (1984) *Wilhelm Roux Arch. Dev. Biol.* **193**, 296–307.
5. Kataoka, T., Powers, S., McGill, C., Fasano, O., Strathern, J., Broach, J. & Wigler, M. (1984) *Cell* **37**, 437–445.
6. Basson, M. E., Thorsness, M. & Rine, J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5563–5567.
7. Goldstein, L. S. B. (1993) *J. Cell Biol.* **120**, 1–3.
8. Higashijima, S., Michiue, T., Emori, Y. & Saigo, K. (1992) *Genes Dev.* **6**, 1005–1018.
9. Poole, S. J., Kauvar, L. M., Drees, B. & Kornberg, T. (1985) *Cell* **40**, 37–43.
10. Coleman, K. G., Poole, S. J., Weir, M. P., Soeller, W. C. & Kornberg, T. (1987) *Genes Dev.* **1**, 19–28.
11. Oro, A. E., Ong, E. S., Margolis, J. S., Posakony, J. W., McKeown, M. & Evans, R. M. (1988) *Nature (London)* **336**, 493–496.
12. Rothe, M., Nauber, U. & Jäckle, H. (1989) *EMBO J.* **8**, 3087–3094.
13. Baumgartner, S., Bopp, D., Burri, M. & Noll M. (1987) *Genes Dev.* **1**, 1247–1267.
14. Côté, S., Priess, A., Haller, J., Schuh, R., Kienlin, A., Seifert, E. & Jäckle, H. (1987) *EMBO J.* **6**, 2793–2801.
15. Rothe, M., Pehl, M., Taubert, H. & Jäckle, H. (1992) *Nature (London)* **359**, 156–159.
16. Gutjahr, T., Patel, N. H., Li, X., Goodman, C. S. & Noll, M. (1993) *Development* **118**, 21–31.
17. Grossniklaus, U., Kurth Pearson, R. & Gehring, W. J. (1992) *Genes Dev.* **6**, 1030–1051.
18. O'Kane, C. J. & Gehring, W. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 9123–9127.
19. Bellen, H. J., O'Kane, C. J., Wilson, C., Grossniklaus, U., Kurth Pearson, R. & Gehring, W. J. (1989) *Genes Dev.* **3**, 1288–1300.
20. Wilson, C., Kurth Pearson, R., Bellen, H. J., O'Kane, C. J., Grossniklaus, U. & Gehring, W. J. (1989) *Genes Dev.* **3**, 1301–1313.
21. Cadigan, K. M., Grossniklaus, U. & Gehring, W. J. (1994) *Genes Dev.* **8**, 899–913.
22. Weigel, D. & Jäckle, H. (1990) *Cell* **63**, 455–456.
23. Clark, K. L., Haley, E. D., Lai, E. & Burley, S. K. (1993) *Nature (London)* **364**, 412–420.
24. Waldorf, U. & Gehring, W. J. (1992) *EMBO J.* **11**, 2247–2259.
25. Rubin, G. M. & Spradling, A. C. (1982) *Science* **218**, 348–353.
26. Karess, R. E. & Rubin, G. M. (1984) *Cell* **38**, 135–146.
27. Frasch, M., Hoey, T., Rushlow, C., Doyle, H. & Levine, M. (1987) *EMBO J.* **6**, 749–759.
28. Krause, H., Klemenz, R. & Gehring, W. J. (1988) *Genes Dev.* **2**, 1021–1036.
29. Tautz, D. & Pfeifle, C. (1989) *Chromosoma* **98**, 81–85.
30. Fjose, A., McGinnis, W. J. & Gehring, W. J. (1985) *Nature (London)* **313**, 284–289.
31. Rijsewijk, F., Schuermann, M., Wagenaar, E., Parren, P., Weigel, D. & Nusse, R. (1987) *Cell* **50**, 649–657.
32. Tabata, T., Eaton, S. & Kornberg, T. (1992) *Genes Dev.* **6**, 2635–2645.
33. Baker, N. E. (1988) *Dev. Biol.* **125**, 96–108.
34. Bejsovec, A. & Martinez-Arias, A. (1991) *Development* **113**, 471–487.
35. Noordermeer, J., Johnston, P., Rijsewijk, F., Nusse, R. & Lawrence, P. A. (1992) *Development* **116**, 711–719.
36. Heemskerk, J., DiNardo, S., Kostriken, R. & O'Farrell, P. H. (1991) *Nature (London)* **352**, 404–410.
37. MacDonald, P. M., Ingham, P. & Struhl, G. (1986) *Cell* **47**, 721–734.
38. Coulter, D. E. & Wieschaus, E. (1988) *Genes Dev.* **2**, 1812–1823.
39. Mohler, J. (1988) *Genetics* **120**, 1061–1072.
40. Kornberg, T. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1095–1099.