Ftz modulates Runt-dependent activation and repression of segment-polarity gene transcription

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Summary

A crucial step in generating the segmented body plan in Drosophila is establishing stripes of expression of several key segment-polarity genes, one stripe for each parasegment, in the blastoderm stage embryo. It is well established that these patterns are generated in response to regulation by the transcription factors encoded by the pair-rule segmentation genes. However, the full set of positional cues that drive expression in either the odd- or even-numbered parasegments has not been defined for any of the segment-polarity genes. Among the complications for dissecting the pair-rule to segment-polarity transition are the regulatory interactions between the different pair-rule genes. We have used an ectopic expression system that allows for quantitative manipulation of expression levels to probe the role of the primary pair-rule transcription factor Runt in segment-polarity gene regulation. These experiments identify sloppy paired 1 (slp1) as a gene that is activated and repressed by Runt in a simple combinatorial parasegment-dependent manner. The combination of Runt and Odd-paired (Opa) is both necessary and sufficient for slp1 activation in all somatic blastoderm nuclei that do not express the Fushi tarazu (Ftz) transcription factor. By contrast, the specific combination of Runt + Ftz is sufficient for slp1 repression in all blastoderm nuclei. We furthermore find that Ftz modulates the Runt-dependent regulation of the segment-polarity genes wingless (wg) and engrailed (en). However, in the case of en the combination of Runt + Ftz gives activation. The contrasting responses of different downstream targets to Runt in the presence or absence of Ftz is thus central to the combinatorial logic of the pair-rule to segment-polarity transition. The unique and simple rules for slp1 regulation make this an attractive target for dissecting the molecular mechanisms of Runt-dependent regulation.

Key words: Runx, Hox, Zic, Segmentation, sloppy paired 1, engrailed, wingless, Drosophila

Introduction

Extensive genetic and molecular studies have elucidated the major principles of the pathway that generates the segmented body plan of the Drosophila embryo. Broad gradients of maternal information are decoded in three successive steps by zygotically expressed segmentation genes, each step occurring with a finer level of spatial resolution. Transcriptional regulation is central to establishing the expression patterns of different segmentation genes at each step. Indeed, it is well established that the final step of the segmentation hierarchy, the pair-rule to segment-polarity transition relies on combinatorial regulation by the pair-rule transcription factors. Furthermore, it is clear that different combinatorial rules are used to generate the odd- and even-numbered stripes of several key segment-polarity genes. However, for none of these segment-polarity genes is there a full understanding of the positional cues responsible for expression of either the odd- or even-numbered stripes. One reason for this is that the transcription factors encoded by the primary pair-rule genes even-skipped (eve), hairy (h) and runt (run – FlyBase), also have important roles in pair-rule gene regulation (Carroll and Scott, 1986; Frasch and Levine, 1987; Ingham and Gergen, 1988; Manoukian and Krause, 1992; Tsai and Gergen, 1994; Tsai and Gergen, 1995).

This complication makes it difficult to identify the exact roles of these three factors in segment-polarity gene regulation and has obscured our understanding of the combinatorial rules underlying the pair-rule to segment-polarity transition.

One pivotal player in the pair-rule to segment-polarity transition is Runt, the founding member of the Runx family of transcription factors. Runx proteins function both as activators and repressors of transcription in multiple developmental pathways (Coffman, 2003; Komori, 2002; Shapiro, 1999; Speck et al., 1999; Wheeler et al., 2000). Indeed, Runt has separable roles in three developmental pathways, sex determination, segmentation and neurogenesis, within the first few hours of Drosophila embryogenesis (Duffy and Gergen, 1994). Ectopic expression experiments indicate a role for Runt in establishing polarity within each parasegment (Manoukian and Krause, 1993). The four-cell wide run stripes overlap the anterior half of each ftz stripe and the posterior half of each eve stripe. The contrasting positive and negative regulatory effects of run on ftz and eve, respectively, contribute to the graded activity of these two genes within each parasegment. However, Runt has additional effects on segment-polarity gene expression beyond modulating ftz and eve expression. For example, the odd-numbered en stripes are repressed by Runt,
even in cells that express Eve (Tracey et al., 2000). The immediate response of en to transient induction of a heat-inducible hs-runT transgene strongly suggests this repression is direct. Additional insights on Runt function have been obtained in other experiments with hs-runT transgenes (Li and Gergen, 1999; Pepling and Gergen, 1995; Tsai et al., 1998). However, the difficulty in reproducibly controlling the precise level and timing of expression makes this approach less than ideal for further dissecting the role of Runt and other pair-rule transcription factors in segment-polarity gene regulation.

We have recently taken advantage of an alternative strategy to investigate the segmentation gene network, and in particular the regulatory functions of Runt. This strategy uses Drosophila lines that maternally express the yeast transcriptional activator GAL4 to drive expression of GAL4-responsive UAS transgenes concomitant with the onset of zygotic transcription during the blastoderm stage of embryogenesis. The transgene construct used to express GAL4 maternally contains the nanos promoter and the 3′ untranslated region of an α-tubulin mRNA and is thus referred to as an NGT transgene (nanos-GAL4-tubulin). Importantly, the expression level can be quantitatively and reproducibly manipulated by using NGT lines that drive different levels of GAL4 expression (Tracey et al., 2000). Experiments with this system have confirmed the potent activity of Runt as a repressor of the odd-numbered en stripes. Indeed the lethality associated with NGT-driven Runt expression has provided the basis for a genetic dissection of en repression (Wheeler et al., 2002).

We have used this approach to systematically examine the responses of pair-rule and segment-polarity genes to different levels of Runt. After en, the second most sensitive segmentation gene target of Runt is the slp1 transcription unit of the sloppy paired locus. We find that the combinatorial rules needed to generate two-cell wide stripes of the segment-polarity genes parasegments. This Runt-dependent activation involves cooperation with the zinc-finger transcription factor encoded by the pair-rule gene opa. Indeed, the simple combination of Runt + Opa is sufficient for slp1 activation in all somatic blastoderm cells that do not have Ftz. We furthermore find that repression of slp1 in the anterior half of the even-numbered parasegments requires Ftz. Ftz not only blocks Runt-dependent activation in these cells, but the combined action of Runt and Ftz is sufficient for slp1 repression in all blastoderm nuclei. Thus, Runt is switched from an activator to a repressor of slp1 by the Ftz homeodomain transcription factor. Additional experiments indicate that Ftz also modulates the activity of Runt on the segment-polarity genes wg and en. However, in the case of en the combination of Runt + Ftz gives activation rather than repression. These results provide important new insights into the context-dependent activity of Runt in segmentation and also provide a valuable framework for dissecting the mechanisms of transcriptional activation and repression by Runt.

Embryo manipulation and in situ hybridization

Embryos were collected as described (Tsai and Gergen, 1994). For experiments with temperature-sensitive mutations, embryos were collected for 1 hour at 25°C, grown for 4.5 hours at the permissive temperature of 18°C, and then shifted to the non-permissive temperature of 30°C for 20 minutes immediately prior to fixation for in situ hybridization.

In situ hybridization with digoxigenin-labeled antisense RNA probes was carried out as described (Klinger and Gergen, 1993) with the following modifications: embryos were digested with Proteinase K (30 μg/ml in PBT=PBS + 0.1% Tween-20) for 3 minutes followed by inactivation with glycine (2 mg/ml) in PBT. To further reduce non-specific binding, embryos were also pre-treated in a 1% (v/v) solution of heat inactivated goat serum for 1 hour prior to incubation with the anti-digoxigenin antibody.

The protocol for double-label in situ hybridization using biotin- and digoxigenin-labeled RNA probes was adapted from that described by O’Neill and Bier (O’Neill and Bier, 1994), with the following modifications: embryos were digested with Proteinase K (50 μg/ml in PBT) for 2.5 minutes; hybridization was carried out at 65°C overnight; post-hybridization washes were carried out in 1% goat serum, 0.3% deoxycholate, 0.3% triton-X in PBS in place of BSA/PBT; and the immunohistochemical detection reaction of HRP-conjugated antibodies with peroxidase and diaminobenzidine was stopped by washing the embryos four times in HRP buffer (50 mM citric acid, 50 mM ammonium acetate, pH 5).

The plasmid templates used to generate digoxigenin-labeled riboprobes for odd-skipped (oda), paired (prd) and slp1 are described in Wheeler et al. (Wheeler et al., 2002). The templates for en and ftz

Materials and methods

Drosophila strains and transgenes

Stocks carrying the opa[1] and ftz[11] mutations were obtained from the Bloomington Stock Center. The temperature-sensitive runT[YP17] mutation (Gergen and Wieschaus, 1986) corresponds to the runT[29] allele listed in the Bloomington Stock Center. The y w[67c23] strain used to generate all transgenic lines was used as the wild-type control strain for in situ hybridization experiments.

The GAL4-drivers P GAL4-nos.NGT11 (NGT11), P GAL4-nos.NGT40 (NGT40) and P GAL4-nos.NGT4A (NGTA) have been described previously (Tracey et al., 2000; Wheeler et al., 2002). Homozygous NGT40 females produce approximately twice the levels of maternal GAL4 activity as females homozygous for either NGT11 or NGTA. Females homozygous for both NGT40 and NGTA produce ~1.5 times more activity than homozygous NGT40 females, whereas females heterozygous for both NGT40 and NGTA produce ~0.75x the activity of homozygous NGT40 females.

The P UAS-runT[14] (UAS-runT[14]), P UAS-runT[15] (UAS-runT[15]) and P UAS-runT[232] (UAS-runT[232]) transgenes have been described previously (Li and Gergen, 1999; Tracey et al., 2000). The third chromosome-linked P UAS-runT[13] (UAS-runT[13]) transgene is comparable in activity with UAS-runT[232]. The P UAS-opa.VZ136 (UAS-opa[36]) transgene insertion was created by standard germ line transformation using the p∆2-3 helper plasmid. This transposon construct was generated by first digesting pNB40-opa[C] (Benedy et al., 1994) with BstEI and BglII to remove sequence containing the SP6 promoter and 5′ untranslated leader of the Xenopus β-globin gene as well as 177 nucleotides of the opa 5′ untranslated leader. The digested plasmid was treated with Klenow polymerase and re-circularized with DNA ligase. A 2.8 kb EcoRI fragment from this modified opa construct was then excised and re-cloned into pUAS-T (Brand and Perrimon, 1993). The second chromosome-linked P UAS-opa.VZ14 (UAS-opa[14]) insertion as well as the third chromosome linked P UAS-opa.VZ10 (UAS-opa[10]) and P UAS-opa.VZ12 (UAS-opa[12]) insertions, were obtained by mobilization of UAS-opa[36] (Robertson et al., 1988). Based on the lethality associated with different levels of NGT-driven expression we estimate that UAS-opa[12], UAS-opa[14] and UAS-opa[10] are expressed at 2.5-, 3- and 4-fold higher levels, respectively, than UAS-opa[36]. The UAS-ftz[261] line was provided by U. Lohr and L. Pick.
are described elsewhere (Tracey et al., 2000; Tsai and Gergen, 1994). The biotinylated ftz riboprobe was synthesized with Biotin-21-UTP (Clontech) in place of digoxigenin-conjugated UTP. The gooseberry (gsb) probe was synthesized with T7 RNA polymerase using SalI-linearized Bsh9c2 (Baumgartner et al., 1987). The hedgehog (hh) probe was synthesized with T3 RNA polymerase using NdeI-linearized pB:hh[4/18.3] (J. Mohler, personal communication). The wg riboprobe was synthesized with T3 RNA polymerase using an EcoRI-linearized pwg-12 template. This pBlueScript plasmid contains a 1.3 kb HindIII + EcoRI genomic fragment that encodes much of the 4th and 5th exons (N. Baker, personal communication).

Results

slp1 is a sensitive target of Runt

The role of Runt as a primary pair-rule gene complicates interpreting the alterations in segment-polarity gene expression that are observed in run mutants. Recent experiments utilizing a GAL4-based NGT-expression system to manipulate expression in the blastoderm embryo demonstrated that low levels of Runt repress en in odd-numbered parasegments without altering expression of the pair-rule genes eve and ftz (Tracey et al., 2000). This observation suggested that this approach might provide a useful tool for defining the role of Runt in regulating other segment-polarity genes. We therefore undertook a systematic survey of the response of other segmentation genes to increasing levels of NGT-driven ectopic Runt expression. These experiments revealed significant differences in sensitivity as well as interesting differences in the nature of the response of different genes to ectopic Runt. As found previously, the odd-numbered en stripes are repressed at both intermediate and high levels of NGT-driven Runt (Fig. 1A-C). After en, the second most sensitive target is slp1. This gene shows a partially penetrant and subtle defect in the spacing of the segmentally repeated stripes in embryos with low levels of NGT-driven Runt (data not shown). A more pronounced alteration is obtained in embryos with intermediate levels of Runt. In these embryos the slp1 pattern is converted from a segment-polarity-like, 14-stripe pattern (Fig. 1D) to a pair-rule-like, seven-stripe pattern (Fig. 1E). At this level, expression of other segmentation genes is normal although there are subtle changes in the spacing of the wg stripes (Fig. 1G,H) and a partial loss of the odd-numbered hh stripes (Fig. 1J,K). All three of these genes show clearer alterations at higher levels of NGT-driven Runt (Fig. 1F,I,L), with wg responding in a

Fig. 1. slp1 is a sensitive target of Runt. In situ hybridization reveals the segmentally repeated expression patterns of different segmentation genes in gastrula stage embryos. Embryos in this and other figures are shown anterior towards the left, dorsal side upwards. Each row shows expression of a different segmentation gene as labeled on the right. The wild-type expression patterns are shown in the left column of embryos. Embryos in the middle and right column of embryos have intermediate and high levels of NGT-driven ectopic Runt expression, respectively. Intermediate and high levels of ectopic Runt were obtained by mating females homozygous for NGT11 and NGT40, respectively to homozygous UAS-runt[232] males. These are the same combinations used to demonstrate that high, but not intermediate, levels of Runt alter expression of eve and ftz (Tracey et al., 2000). Not shown in this panel are the responses of the pair-rule genes hairy and odd-paired (opa). NGT-driven Runt expression leads to stripe-specific repression of hairy similar to that obtained in hs-runt embryos, but only at high levels of expression. The opa pattern is unique amongst the pair-rule genes and is expressed in a broad band spanning the pre-segmental region of the embryo, rather than in a series of stripes. This pattern is not altered by ectopic Runt.
manner similar to slp1 and hh responding in a manner similar to en. High Runt levels also produce spacing defects in the expression of odd and gsb, as well as a more subtle effect on prd (Fig. 10, R, U). Several of the changes observed at high levels of ectopic Runt are likely to be indirect and due to alterations in the expression of eve, ftz and hairy (Tracey et al., 2000; Tsai and Gergen, 1994; Tsai and Gergen, 1995). The response of slp1 to ectopic Runt is notable both because of its sensitivity and apparent simplicity, thus suggesting that Runt plays a pivotal role in regulating slp1 transcription.

Parasegment-specific activation and repression of slp1 transcription by Runt

slp1 is expressed in a repeating two-cell wide stripe pattern in late blastoderm stage embryos (Fig. 2A). The phasing of this expression relative to other key pair-rule genes is shown in Fig. 2B. Parasegmental units are defined by expression of the homeodomain proteins Eve and Ftz. The initial pair-rule expression of Eve and Ftz is in complementary, four cell wide stripes. These stripes narrow during the process of cellularization as expression is lost in the more posterior cells in each parasegment. The slp1 stripes arise in the two most posterior cells in each parasegment, the cells that are first to lose Eve and Ftz expression. Interestingly, the borders of the slp1 stripes within each parasegment align with the borders of run and hairy expression. These two pair-rule genes are expressed in complementary patterns that are shifted by two cells relative to the eve and ftz patterns (Ingham and Gergen, 1988; Kania et al., 1990; Kosman et al., 1998). In odd parasegments, slp1 is activated in cells that express Runt, whereas in even parasegments slp1 is repressed in the Runnt-expressing cells. As will be shown below, slp1 activation in odd-numbered parasegments requires the specific combination of Runt and Opa, whereas repression in even-numbered parasegments involves the specific combination of Runt + Ftz. These two regulatory interactions are schematically depicted in Fig. 2B to provide a framework for interpreting the alterations produced by the various genetic manipulations described below.

The expression of slp1 in embryos deficient for Runt consists of six irregularly spaced stripes of different widths and intensities (data not shown). These several changes reflect the altered expression of other pair-rule genes in these embryos. In order to more specifically define the role of Runt in slp1 regulation we took advantage of the temperature-sensitive run1[YP17] mutation. Embryos hemizygous for this mutation were allowed to develop at the permissive temperature through the early blastoderm stage when the seven-stripe patterns of the pair-rule genes are established. These embryos were then shifted to the non-permissive temperature for 20 minutes and then fixed for in situ hybridization. This transient elimination of run leads to loss of slp1 expression in odd-numbered parasegments (Fig. 2C). There is also expanded slp1 expression in even-numbered parasegments, with the exception that expression is lost in the ventral portion of parasegment 4 (Fig. 2C). The contrasting loss of activation and partial loss of repression produced by this transient reduction of run is schematically depicted in Fig. 2D. These results demonstrate an acute temporal requirement for Runt in slp1 regulation and strongly suggest that Runt normally functions as both an activator and repressor of slp1 transcription.

As described above, the 14-striped slp1 pattern is converted into a seven-stripe pattern in the presence of intermediate as well as high levels of NGT-driven Runt (Fig. 1E,F). These seven stripes are broader than the two-cell wide stripes that normally comprise the posterior half of each parasegment. We used double in situ hybridization to investigate the relationship between these broadened slp1 stripes and the expression of ftz, which identifies cells in even-numbered parasegments. The function of Runt as an activator of ftz is revealed by broadened four-cell wide stripes in gastrula stage embryos that have high
levels of NGT-driven Runt (Fig. 2E). In wild-type embryos the ftz mRNA pattern is resolved to 2 cell-wide stripes by this stage. The broadened stripes of slp1 and ftz in these embryos are complementary to each other (Fig. 2E). Thus, uniform expression of Runt in the blastoderm embryo leads to activation of slp1 in all cells within odd parasegments while conversely leading to repression in all cells within even parasegments (Fig. 2F). The changes produced at these high levels of Runt may in part be indirect. Indeed, as will be shown below, the broadening of ftz contributes to the repression of slp1 in even-numbered parasegments. Nevertheless, this result provides compelling evidence that Runt has a dual, parasegment-specific role in slp1 regulation.

**Runt and Opa cooperate to activate slp1 transcription**

Based on the above results we examined the role of all of the other pair-rule genes in slp1 regulation. A somewhat surprising result from these experiments is that slp1 expression in odd-numbered parasegments is lost in opa mutant embryos (Fig. 3B). The importance of Opa is surprising as expression of other segment-polarity genes is reduced, but not eliminated in opa mutants (Benedyk et al., 1994; Cimbroa and Sakonju, 1995). Moreover, Opa is expressed at uniform levels throughout the pre-segmental region of the embryo, and thus does not provide positional information that defines the placement of slp1 stripes relative to other pair-rule transcription factors. As shown above, the odd-numbered slp1 stripes require Runt, and are interpreted to expand in response to ectopic Runt. We tested the requirement for Opa in this Runt-dependent activation by examining slp1 expression in embryos that have high levels of NGT-driven Runt and that are also mutant for opa. Expression of slp1 within the pre-segmental region is lost in these embryos (Fig. 3E). This result corroborates our interpretation that the expanded slp1 stripes produced by NGT-driven Runt correspond to the odd-numbered stripes and further confirms the importance of Opa for Runt-dependent activation.

A useful feature of the GAL4 expression system is that expression levels can be varied by changing the strengths of either the GAL4 driver, or the responding UAS transgene (e.g. Li and Gergen, 1999). We took advantage of this feature to further investigate the relative roles of Runt and Opa in slp1 regulation by generating a co-expression matrix with a panel of different UAS-runt and UAS-opa lines. Increasing the level of Opa in embryos with the same low level of NGT-driven Runt (Fig. 4A-C) alters slp1 in a manner similar to that obtained by increasing Runt alone (compare Fig. 4C with Fig. 1F). Thus, Opa potentiates Runt-dependent regulation in a concentration-dependent manner. Concentration-dependent effects of Opa are also observed at both intermediate (Fig. 4D-F) and high (Fig. 4G-I) levels of NGT-driven Runt. In order to interpret these changes, it is useful to first consider the relatively simple, yet striking response of slp1 to high levels of both Runt and Opa (Fig. 4I). In these embryos, slp1 is expressed throughout the anterior head region and is nearly uniformly repressed throughout the pre-segmental region of the embryo. The anterior activation is particularly informative as none of the other pair-rule or segment-polarity gene shows this response to anterior activation is particularly informative as none of the other pair-rule or segment-polarity gene shows this response to anterior activation coupled with this mutual dose-dependent cooperation strongly suggests that these two factors function together in a concentration-dependent complex to activate slp1 transcription.

The other notable response to high levels of Runt and Opa is the nearly complete repression of slp1 throughout the presegmental region of the embryo (Fig. 4I). As described above, slp1 and ftz are expressed in complementary patterns in
Ftz and Runt cooperate to repress slp1
plays a key role in

Moreover, these results strongly suggest that Ftz
observations indicate that Opa potentiates the ability of Runt
Variations
NGTA
and
obtained in crosses using
Runt and Opa. In all cases,
etropic expression was
females homozygous for both
NGT40 and NGTA. Variations
expression were obtained
using different UAS-runt and
UAS-opa lines. Embryos in
the first row carry UAS-runt[14] in
combination with (A) UAS-
opa[36], (B) UAS-opa[12] and
(C) UAS-opa[10], which are
ordered in increasing strength
from left to right. Embryos in
the second row (D-F) carry
UAS-runt[232] in combination
with the same three UAS-opa
transgenes. Embryos in the
third and fourth rows (G-L)
carry UAS-runt[15], also in combination with the same three UAS-opa transgenes. The different levels of ectopic Runt expression are organized in increasing strength from top to bottom, UAS-runt[232] and UAS-runt[15], giving approximately two- and fivefold increases, respectively, over the level obtained with UAS-runt[14] (Li and Gergen, 1999).

Runt expression is unaltered in these embryos (data not shown). Thus, Ftz-dependent repression of slp1 occurs only in cells that express Runt (Fig. 5F). The observation that slp1 expression in even-numbered parasegments is resistant to repression by Ftz indicates that some other factor is required for Ftz-dependent repression. Ftz normally represses slp1 in Runt-expressing cells in even-numbered parasegments and ectopic Ftz leads to repression in the Runt-expressing cells in odd parasegments. This correlation strongly suggests that slp1 is repressed by the specific combination of Runt + Ftz. We tested this hypothesis using the NGT system to express both Runt and Ftz throughout the embryo. Consistent with our hypothesis, co-expression of Runt and Ftz represses slp1 throughout the embryo (Fig. 5G). This result provides a clear indication of the ability of Runt to repress slp1. Moreover, the reciprocal effects produced in the absence (Fig. 5C) versus presence of Ftz (Fig. 5G) provide compelling evidence that Ftz converts Runt from an activator to a repressor of slp1 transcription.

Ftz regulates the activity of Runt on the segment-polarity genes wg and en
The above experiments were initiated due to the sensitivity and simplicity of the slp1 response to NGT-driven Runt. Although wg is less sensitive than slp1, the parallel responses of these two genes (Fig. 1F,I) suggest that Ftz and Runt interact in a similar manner to regulate wg. The one cell-wide wg stripes identify the posterior-most cells within each parasegment and correspond to a subset of the slp1-expressing cells (Fig. 6). As observed for slp1, transient elimination of run leads to loss of wg expression in odd-parasegments and expanded expression in a subset of even-numbered parasegments (Fig. 7A).
Fig. 5. Ftz converts Runt from an activator to a repressor of slp1. (A) Embryos mutant for ftz express slp1 in the anterior half of the even-numbered parasegments, resulting in six-cell wide stripes. (B) The ftz mutant phenotype and the way in which this expanded expression is accounted for by Runt+Opa dependent activation. Note that run expression is not significantly altered in ftz mutant embryos at this stage (Klingler and Gergen, 1993). (C) Ectopic Runt expression in ftz mutant embryos activates slp1 throughout the pre-segmental region. This embryo was obtained from a cross between a female heterozygous for NGT40, NGTA and the ftz[11] mutation with a male homozygous for UAS-runt[232] and heterozygous for ftz[11]. The level of NGT-driven Runt obtained with this combination does not fully overcome Eve-dependent repression, resulting in a few thin stripes of cells with reduced slp1 expression. Runt+Opa-dependent activation (D) results in the slp1 pattern shown in C. The effects of NGT-driven Ftz on slp1 is shown in E and interpreted in F. The embryo in E is from a mating between homozygous NGT40 females and homozygous UAS-ftz[261] males. The effects of co-expressing Runt and Ftz are shown in G and interpreted in H. The embryo in G is from a mating between homozygous NGT40 females and males homozygous for both UAS-runt[232] and UAS-ftz[261].

Odd-numbered wg stripes are specifically repressed by NGT-driven Ftz expression (Fig. 7B), whereas co-expression of Runt and Ftz represses wg in both odd- and even-numbered parasegments (Fig. 7C). Thus, as found for slp1, Runt and Ftz specifically cooperate to repress wg. However, the rules for Runt-dependent activation of wg are more complex than for slp1, as NGT-driven co-expression of Runt and Opa is not sufficient for wg activation in the anterior unsegmented region of the embryo (Fig. 7D). Although the full set of rules for wg regulation thus remains elusive, these results demonstrate a pivotal role for Ftz in modulating the regulatory effects of Runt on wg expression.

Similar experiments indicate that specific interactions between Ftz and Runt are important for regulation of the segment-polarity gene en. However, the striking result in this case is that Runt and Ftz cooperate to activate, rather than repress transcription. en is normally expressed in the anterior most row of cells in each parasegment (Fig. 6). The even-numbered stripes arise in Ftz-expressing cells and require Ftz for expression (DiNardo and O’Farrell, 1987; Florence et al., 1997). Runt also plays an acute role in activation of the even-numbered en stripes as they are lost in response to a transient reduction in run (Fig. 7E). Ectopic expression experiments provide further evidence that the specific combination of Runt + Ftz gives activation of en. NGT-driven Ftz leads to expansion of the even-numbered en stripes (Fig. 6).
Fig. 7. Ftz modulates Runt-dependent activation and repression of wg and en. Expression of wg (A-D) and en (E-H) in response to different perturbations in Runt and Ftz activity. (A,E) Response of these two genes to transient elimination of run in a temperature-shifted run[YP17] embryo. (B,F) Response to NGT-driven Ftz. These embryos are from a mating of homozygous NGT40 females to homozygous UAS-fzt[261] males. (C,G) NGT-driven co-expression of Runt and Ftz blocks wg expression, while producing a pair-rule pattern of en expression. (D) Response of wg to NGT-driven co-expression of Runt and Opa. This embryo is from a mating of females homozygous for both NGT40 and NGTA to males homozygous for the UAS-runt[13] and UAS-opa[14] transgenes. This combination results in a slp1 pattern similar to the embryos in Fig. 4F,H. (H) Response of en to NGT-driven Runt in embryos that are also homozygous for the ftz[11] mutation. This embryo is from the same cross used to generate the embryo in Fig. 5C.

7F), presumably into cells that normally express Runt but not Ftz. More provocative is the pair-rule like expression of en in response to NGT-driven co-expression of both Runt and Ftz (Fig. 7G). The en activation in these embryos is Ftz dependent as all expression is lost in ftz mutant embryos that have the same levels of NGT-driven Runt (Fig. 7H). The observation that co-expression of Runt and Ftz does not produce activation in the head suggests that another spatially restricted factor also participates in en activation. The non-uniform expression of en in the pre-segmental region of these embryos could be due to non-uniform expression of this additional activator and/or to regulatory inputs from a repressor such as odd (Mullen and DiNardo, 1995). Thus, as found for wg, additional regulatory inputs are used to refine the response of en to Runt and Ftz. These complications aside, the contrasting response of en to NGT-driven Runt in the presence (Fig. 7G) versus absence (Fig. 7H) of Ftz provides compelling evidence that Ftz plays a pivotal role in modulating the Runt-dependent regulation of en. Taken together with the results on slp1 and wg, these experiments indicate that the combination of Runt + Ftz is interpreted in a gene-specific manner to give either activation or repression of segment-polarity gene transcription.

Discussion

slp1 regulation and the pair-rule to segment-polarity transition

The differential combinatorial effects of Runt and Ftz on segment-polarity gene regulation described above emerged as a result of analyzing the sensitive and relatively simple response of slp1 to ectopic Runt. The slp1 transcription unit is one of two redundant genes that comprise the slp locus (Grossniklaus et al., 1992). This locus was initially characterized as having a pair-rule function in the segmentation gene hierarchy based on a weak pair-rule phenotype associated with loss of slp1 function (Grossniklaus et al., 1992; Nusslein-Volhard et al., 1984). The slp1 and slp2 genes are expressed in similar patterns during early embryogenesis. Embryos deficient for both slp1 and slp2 have an unsegmented lawn cuticle phenotype similar to that produced by wg mutations (Grossniklaus et al., 1992). This raises the question of whether it is most appropriate to consider slp as a pair-rule or segment-polarity locus. In the most straightforward interpretation of the segmentation hierarchy, the role of the pair-rule genes is to establish the initial metameric expression patterns of the segment-polarity genes. The initial expression of the key segment polarity genes en and wg is normal in gastrula stage embryos that are deleted for both slp1 and slp2 (data not shown). The expression of wg begins to become abnormal and is lost during early germband extension. These observations are consistent with the proposal of Grossniklaus and colleagues that slp expression identifies cells that are competent to maintain wg expression subsequent to the blastoderm stage (Cadigan et al., 1994). Based on these observations, we conclude that slp1 and slp2 are most appropriately classified as segment polarity genes, not pair-rule genes.

The expression of slp1 (and slp2) differs from several other segment-polarity genes in that the metameric pattern is comprised of two-cell wide, rather than single-cell wide stripes. These two cell-wide stripes comprise the posterior half of each parasegment (Fig. 6). As shown above, slp1 activation in odd-numbered parasegments requires the cooperative action of Runt and Opa, whereas in even-numbered parasegments Runt works together with Ftz to repress slp1 expression. The simple rules involving these three factors fully account for slp1 regulation in all of the Runt-expressing cells in the blastoderm embryo (Fig. 6) but also raise a question regarding the positional cues that regulate slp1 expression in cells that do not express Runt.
There are four other pair-rule transcription factors that could be involved in \textit{slp1} regulation: Eve, Hairy, Odd and Prd. The phasing of the pair-rule expression domains of these factors are shown in Fig. 6. Expression of both Odd and Prd overlaps the \textit{slp1} stripes in a manner that suggests that neither of these factors provides positional information crucial for \textit{slp1} regulation. Consistent with this, there are no substantial changes in the early 14-striped \textit{slp1} pattern in embryos mutant for either \textit{odd} or \textit{prd} (data not shown). By contrast, elimination of either Eve or Hairy leads to changes in both the number and spacing of the \textit{slp1} stripes. However, as these are both primary pair-rule genes some of these changes are certainly indirect and due to alterations in Runt and Ftz expression (Carroll and Scott, 1986; Ingham and Gergen, 1988; Klingler and Gergen, 1993).

Several lines of evidence indicate that Eve has a direct role in \textit{slp1} repression. Experiments with the temperature-sensitive \textit{eve}[ID19] mutation indicate that transient elimination of Eve at the cellular blastoderm stage leads to expanded six cell-wide \textit{slp1} stripes because of de-repression in the anterior two cells of each odd-numbered parasegment (data not shown). These two are the cells with the highest level of Eve, indicating that the primary role of Eve at this stage is to repress \textit{slp1} expression (Fig. 6). Complementary experiments with an inducible \textit{hs-Eve} transgene reveal that ectopic Eve blocks \textit{slp1} activation in both odd- and even-numbered parasegments (data not shown). This result not only confirms Eve’s role as a repressor, but also reveals a crucial difference between Eve- and Ftz-dependent repression. As shown above, Ftz-dependent repression is restricted to odd-numbered parasegments unless Runt is also ectopically expressed (Fig. 5). This same restriction is observed in experiments with \textit{hs-Ftz} transgenes (data not shown), indicating that the difference between Eve and Ftz is not due to the mode of ectopic expression. Taken altogether these results indicate that Eve and Ftz normally have comparable roles in repressing \textit{slp1} transcription in the anterior half of the odd- and even-numbered parasegments, respectively, in late blastoderm stage embryos (Fig. 6). The key distinction in the regulation of \textit{slp1} by these two homeodomain transcription factors is the critical role that Runt plays in Ftz-dependent repression.

One aspect of \textit{slp1} expression not accounted for by the above rules is the factor (or combination of factors), referred to here as factor X, that is responsible for \textit{slp1} activation in the posterior half of the even-numbered parasegments (Fig. 6). Activation in these cells is blocked either by the combination of Runt+Ftz (Fig. 5G) or by ectopic Eve (data not shown). Runt and Ftz are co-expressed anterior to these even-numbered stripes and presumably play a role in defining the anterior margin of these stripes. Conversely, Eve is expressed posterior to these cells and probably has a role in defining the posterior margins of these stripes. The sole pair-rule transcription factor that remains as a candidate for Factor X is Hairy, which is expressed in the posterior half of even-numbered parasegments (Fig. 6). However, we do not think that factor X is Hairy for several reasons. All of the evidence to date indicates that Hairy functions as a repressor (Barolo and Levine, 1997; Ish-Horowicz and Pinchin, 1987; Jimenez et al., 1997; Poortinga et al., 1998; van Doren et al., 1994). Furthermore, \textit{NGT}-driven expression of Hairy does not lead to \textit{slp1} activation in anterior blastoderm cells similar to that produced by the co-expression of Runt and Opa (data not shown). Identification of factor X is clearly important for a complete understanding of \textit{slp1} regulation.

**Context-dependent activities of Runt**

Previous studies indicated that Runt has roles in both activating and repressing transcription of different target genes in the \textit{Drosophila} embryo (Kramer et al., 1999; Tracey et al., 2000; Tsai and Gergen, 1994; Tsai and Gergen, 1995). The results presented above provide additional compelling evidence for this dual activity and also provide insight on factors that contribute to this context-dependent regulation. The dramatic effects of Ftz on Runt-dependent \textit{slp1} regulation clearly demonstrate that one important component of context is the specific combination of other transcription factors that are present in a cell. Indeed, the unique and relatively simple rules for \textit{slp1} regulation make this an especially attractive target for dissecting the molecular mechanisms whereby Ftz converts Runt from an activator to a repressor of transcription. It seems likely that the rules governing the Runt-dependent regulation of \textit{slp1} will provide a foundation for understanding the regulation of \textit{wg} and \textit{gsb}, two segment-polarity genes that are expressed in a subset of \textit{slp1}-expressing cells and that respond to Runt in a manner similar, but not identical to \textit{slp1}.

Our results also point to a second important component of context-dependent regulation by Runt. The specific combination of Runt + Ftz, which represses \textit{slp1}, does not always give repression as these same two factors work together to activate \textit{en} in some of these same cells at the same stage of development. Thus, cellular context alone cannot fully account for the regulatory differences and there must be a target-gene specific component of context-dependent regulation. A similar gene-specific example of context-dependent regulation has recently been described for the Runx protein Lozenge (Canon and Banerjee, 2003). In this case, the presence of binding sites for the Cut homeodomain protein helps to stabilize a complex that leads to repression of \textit{deadpan} transcription in the same cells in which Lozenge is responsible for activation of \textit{Drosophila Pax2}. In a strict parallel of this model, we would speculate that the \textit{slp1} regulatory region contains binding sites for some factor that helps to stabilize a repressor complex that includes the Runt and Ftz proteins. In a reciprocal, and not mutually exclusive model, perhaps there are binding sites for a factor in the \textit{en} regulatory region that helps to stabilize a Runt- and Ftz-dependent transcriptional activation complex. Further studies on the \textit{en} and \textit{slp1} cis-regulatory regions are needed in order to address these questions at the molecular level. This future work is crucial for understanding the context-dependent activity of Runt and thus the molecular logic of the control system that underlies the pair-rule to segment-polarity transition in \textit{Drosophila} segmentation.

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