Transcription elongation controls cell fate specification in the *Drosophila* embryo

Xiaoling Wang,[1,2] Chanhyo Lee,[3]
David S. Gilmour,[3] and J. Peter Gergen[1,4]

[1]Department of Biochemistry and Cell Biology and the Center for Developmental Genetics, Stony Brook University, Stony Brook, New York 11794, USA; [2]Graduate Program in Biochemistry and Structural Biology, Stony Brook University, Stony Brook, New York 11794, USA; [3]Center for Gene Regulation, Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania 16802, USA

The simple combinatorial rules for regulation of the *sloppy-paired-1* (*slp1*) gene by the pair-rule transcription factors during early *Drosophila* embryogenesis offer a unique opportunity to investigate the molecular mechanisms of developmentally regulated transcription repression. We find that the initial repression of *slp1* in response to Runt and Fushi-tarazu (Ftz) does not involve chromatin remodeling, or histone modification. Chromatin immunoprecipitation and in vivo footprinting experiments indicate RNA polymerase II (Pol II) initiates transcription in *slp1*-repressed cells and pauses downstream from the promoter in a complex that includes the negative elongation factor NELF. The finding that NELF also associates with the promoter regions of *wingless* (*wg*) and *engrailed* (*en*), two other pivotal targets of the pair-rule transcription factors, strongly suggests that developmentally regulated transcriptional elongation is central to the process of cell fate specification during this critical stage of embryonic development.

The *Drosophila* embryo provides a powerful model for investigating the regulatory mechanisms of cell fate specification during development. Gradients of maternally provided positional information are translated into differential programs of gene expression at a single-cell level of resolution within the 6000 cells that comprise the cellular blastoderm stage embryo at 3 h of development (Ingham 1988). On the antero–posterior axis, this initial specification is revealed by the segmentally repeated expression of several segment-polarity genes (Akam 1987). These metameric expression patterns are generated in response to positional cues provided by the DNA-binding transcription factors encoded by the pair-rule segmentation genes (Howard 1990). One key component of segment-polarity gene regulation is context-dependent activation and repression by the pair-rule transcription factor Runt, the founding member of the Runx family of developmental regulators (Coffman 2003; Levanon and Groner 2004). Recent studies reveal that Runt activity is modulated by the Ftz homeodomain protein and further identify the *slp1* gene as an attractive model for investigating the molecular mechanisms of Runt-dependent transcriptional regulation (Swantek and Gergen 2004).

The segmentally repeated expression of *slp1* initiates during the late cellular blastoderm stage and becomes fully apparent by the onset of gastrulation (~3.5 h of development). The combinatorial rules that generate this pattern differ in odd- and even-numbered parasegments. Expression in the posterior half of odd parasegments requires the pair-rule transcription factors Runt and Odd-paired (Opa). In contrast, in the adjacent cells that comprise the anterior half of the even parasegments, the presence of Ftz converts Runt from an activator to a repressor of *slp1* expression (Swantek and Gergen 2004). Normally, these combinatorial rules only apply within the presegmental region of the embryo. However, ectopic coexpression of Runt and Opa in embryos that are mutant for Ftz leads to *slp1* activation in all somatic blastoderm cells, whereas ectopic coexpression of both Runt and Ftz gives *slp1* repression throughout the embryo (Fig. 1A). Thus, all late blastoderm stage nuclei can adopt *slp1* expression states characteristic of posterior odd-numbered or anterior even-numbered parasegments in response to these pair-rule transcription factors. The uniform repression of *slp1* in response to Runt and Ftz is of particular interest, as it is straightforward to generate staged populations of these embryos that can be used to investigate the in vivo biochemistry of this repression. Indeed, in matched embryo collections processed 3–4 h AED (after egg deposition), quantitative RT–PCR reveals 40-fold less *slp1* mRNA in embryos that are ectopically coexpressing Runt and Ftz compared with wild-type embryos. Our goal in this study was to identify the molecular changes at the *slp1* locus that account for this 40-fold difference in expression.

Results and Discussion

We began by using DNase I hypersensitivity to probe the chromatin structure of the *slp1* locus. These assays revealed the presence of a DNase I-hypersensitive site near to the 5′-end of the *slp1* transcription unit (Fig. 2A). Chromatin immunoprecipitation (ChIP) experiments with antiserum against histone H3 provide an explanation for this DNase I hypersensitivity. There is significantly reduced association of H3 with the *slp1* promoter region compared with both the structural gene as well as sequences upstream of the promoter (Fig. 2B,C). These observations strongly suggest that the promoter region is nucleosome free. Importantly, matched collections of wild-type and Runt + Ftz (R+F) embryos show both the same pattern of DNase I hypersensitivity (Fig. 2A) and histone H3 association (Fig. 2C). These results indicate that the 40-fold decrease in mRNA expression in *slp1*-repressed embryos is not due to gross changes in the accessibility of the *slp1* promoter region.

Histone acetylation and deacetylation are important for transcriptional regulation with a general correlation
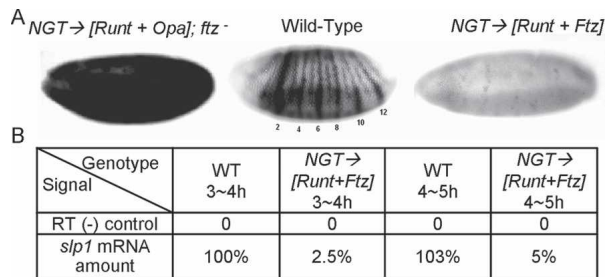
Figure 1. Manipulation of *slp1* expression by pair-rule transcription factors. (*A*) Expression of *slp1* mRNA in gastrula-stage embryos as revealed by in situ hybridization. (*Middle* panel) The wild-type pattern shows 14 distinct stripes in the presegmental region with stronger expression in even-numbered parasegments. The embryos on the *left* and *right* demonstrate the response of *slp1* to ectopic expression of different pair-rule transcription factors using the *NGT* (*nanos-GAL4-tubulin*) expression system (Tracey et al. 2000). (*Left* panel) *NGT*-driven expression of Runt and Opa in embryos that are mutant for *ftz* leads to activation throughout the presegmental region as well as in the anterior head region. (*Right* panel) In contrast, *NGT*-driven coexpression of Runt and Ftz leads to uniform *slp1* repression. (*B*) Quantitative RT–PCR was used to determine the differences in *slp1* expression in staged collections of wild-type (WT) and Runt + Ftz (R+F) coexpressing embryos.

between histone acetylation and active transcription (Roth et al. 2001; Schubeler et al. 2004). Indeed, prior work from our laboratory demonstrated that the *Rpd3* histone deacetylase is important for maintaining the Runt-dependent repression of the segment-polarity gene *en* (Wheeler et al. 2002). ChIP experiments reveal no significant difference in the H3 acetylation pattern of *slp1* chromatin from wild-type versus R+F embryos (Fig. 2D). Although we detect no differences in H3 or Ac-H3 association that correlate with *slp1* repression, there are interesting differences in the H3 acetylation levels at different genomic locations. The *slp1* structural gene (primer pairs 6 and 7) shows stronger Ac-H3 association than the upstream region (primer pair 2) (Fig. 2D). This difference is not observed for the association of H3 with these same intervals (Fig. 2C), suggesting that H3 acetylation marks genomic regions that are permissive for transcription. The relative levels of H3 and Ac-H3 association with *Brother* (*Bro*), a gene that is not transcribed in the early embryo (as measured by RT–PCR) (data not shown), provide additional evidence for this trend. Although H3 association with the *Bro* gene is greater than for any region of the *slp1* locus, the level of Ac-H3 association with *Bro* is lower than for any region of *slp1* (Fig. 2C,D). Based on the observation that we can detect differences in H3 and Ac-H3 association that correlate with transcriptional potential and yet detect no differences between wild-type and *slp1*-repressed embryos, we conclude that H3 acetylation plays a negligible role in the establishment of *slp1* repression.

The above observations led us to characterize the interactions of the transcriptional machinery with *slp1*. Association of the TATA-box-binding protein (TBP) is a first step in assembly of the transcriptional machinery on a promoter (Green 2000). As expected, TBP association is detected with a promoter-proximal interval centered 6 base pairs (bp) upstream of the *slp1* transcript initiation site in chromatin from wild-type embryos (Fig. 3A). A weaker signal is detected for an interval within the 5′ untranslated region (UTR), centered 124 bp down-
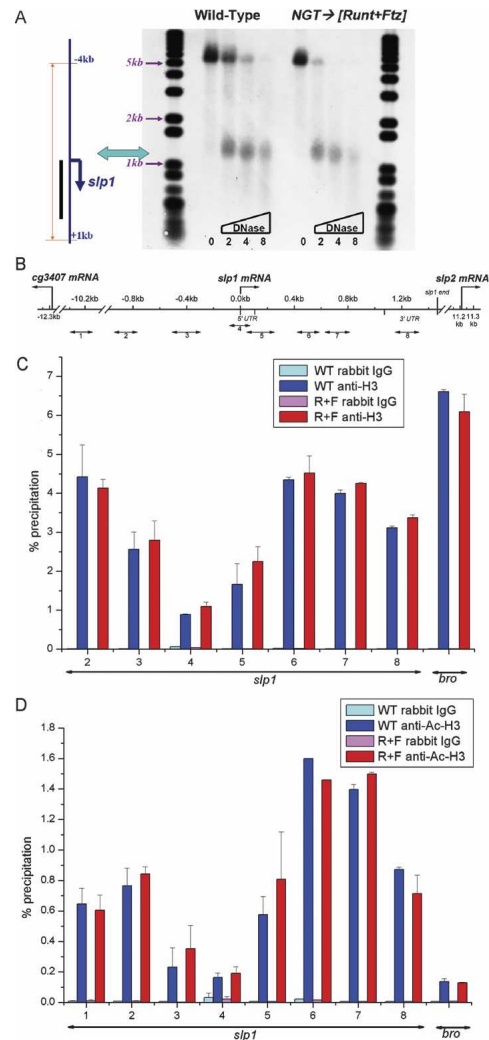

Figure 2. *slp1* repression is established without alterations in chromatin structure. (*A*) DNase I digestion reveals the presence of a hypersensitive site near the 5′-end of the *slp1* transcription unit. The diagram on the *left* indicates the relationship of an ~5-kb interval of the *slp1* locus to the DNA fragments detected in the Southern blot shown on the *right*. The thin line on the *left* indicates the extent of a 5-kb fragment produced by BstXI digestion, and the adjacent thick vertical bar indicates the antisense riboprobe used to detect *slp1*-containing sequences. The horizontal line and downward arrow indicate the initiation site and direction of transcription, respectively. DNA samples isolated from wild-type and R+F embryos (3–4 h AED) are organized as indicated across the *top*, with the amounts of DNase I used on different samples indicated across the *bottom*. (*B*) The location of PCR products generated with different primer pairs used for ChIP studies is shown relative to a map of the *slp1* locus. The PCR products are numbered *left* to *right*, from 1 to 8. (*C*) Results of quantitative PCR on ChIP samples generated using an antibody against histone H3 with chromatin isolated from wild-type (blue bars) and R+F (red bars) embryos. The nonspecific background was determined using a control rabbit IgG antiserum with these same chromatin preparations (cyan and magenta bars, respectively). Results obtained for different intervals of the *slp1* locus are as labeled across the *bottom*. ChIP results using a primer pair that amplifies a segment of the *Bro* structural gene are given on the *right*. Error bars indicate the standard error in the percent precipitation values for each interval. (*D*) Results of quantitative PCR with ChIP samples generated using an antibody that detects acetylated H3, labeled as in *C*.
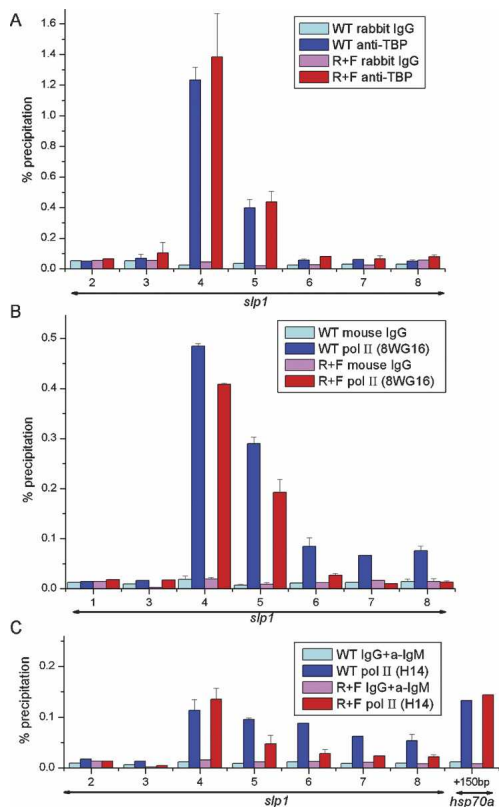
Figure 3. Initiation of transcription in *slp1*-repressed embryos. ChIP assays on chromatin from wild-type and R+F embryos using anti-TBP antiserum (*A*), the monoclonal antibody 8WG16 (*B*), which recognizes the heptad repeats of the CTD, irrespective of their phosphorylation status (Patturajan et al. 1999; Jones et al. 2004; Morris et al. 2005), and the monoclonal antibody H14 (*C*), which specifically recognizes phosphorylation of serine residue 5 within the heptad repeats that comprise the CTD (Patturajan et al. 1999; Jones et al. 2004; Morris et al. 2005). Background controls for each antibody were determined using nonimmune serum from the appropriate species as indicated. The labeling scheme is the same as used in Figure. 2.

stream from the start site, whereas all other intervals give background level signals. We find very similar levels of TBP association in chromatin from R+F embryos (Fig. 3A). More surprising is the finding that there is almost no difference in the level of Pol II association with the *slp1* promoter-proximal interval in chromatin from wild-type and R+F embryos (Fig. 3B). Pol II is also associated with the *slp1* structural gene in wild-type embryos, but at lower levels than at the promoter (Fig. 3B). In contrast, Pol II association with the *slp1* structural gene is markedly reduced in R+F embryos and near to background levels for regions downstream from the 5'-UTR (Fig. 3B). Based on these results, we conclude that promoter recruitment of Pol II is not blocked in *slp1*-repressed embryos. We further characterized *slp1*-associated Pol II using an antibody that recognizes the Phospho-Ser-5 form of the heptad repeats that comprise the C-terminal domain (CTD) of the largest Pol II subunit. Phospho-Ser-5 modification of the CTD is associated with transcription initiation (Komarnitsky et al. 2000; Sims et al. 2004; Morris et al. 2005). This antiserum also gives the strongest signals with the *slp1* promoter-proximal interval in wild-type chromatin, and this signal is not reduced in

chromatin from R+F embryos (Fig. 3C). This result indicates that *slp1* repression occurs at a step downstream from transcription initiation.

The *Drosophila hsp70a* promoter is an extensively studied example of regulated transcriptional elongation (Rougvie and Lis 1988; Boehm et al. 2003; Lebedeva et al. 2005). Pol II initiates transcription at the *hsp70a* promoter, and then, in the absence of a heat shock, pauses immediately downstream from the promoter. All somatic cells in 3–4-h-AED embryos are capable of activating the *hsp70a* gene, and as expected, Phospho-Ser-5-modified Pol II is readily detected on the *hsp70a* promoter in our chromatin preparations from non-heat-shocked embryos (Fig. 3C). The paused Pol II complex on the *hsp70a* promoter is also readily detected using permanganate footprinting due to the increased sensitivity of thymine residues in single-stranded regions (Weber et
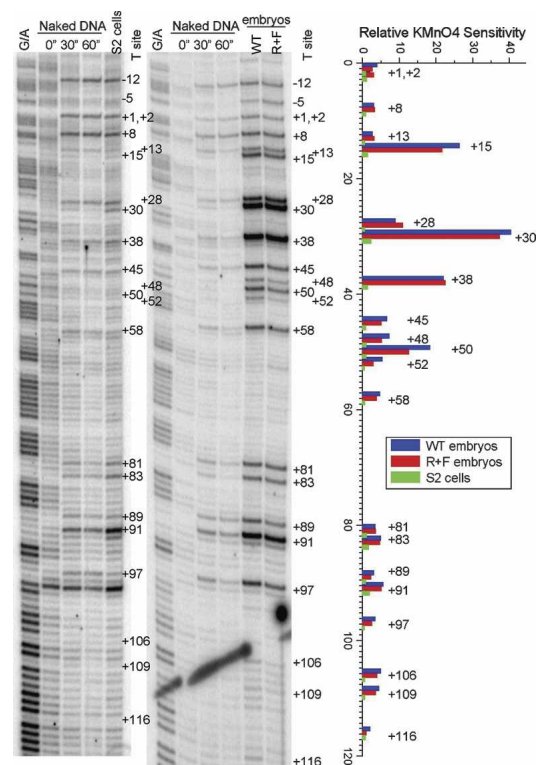

Figure 4. Developmental regulation of *slp1* promoter-proximal pausing. The two *left* panels are autoradiographs revealing the permanganate sensitivity of thymine residues in an interval extending from 12 nt upstream of to 116 nt downstream from the *slp1* transcription start site. Each of these panels includes a G/A ladder and three lanes showing results obtained by treating naked DNA with 10 mM KMnO₄ for 0, 30, and 60 sec. In addition, the autoradiograph on the *left* shows in vivo results obtained from treatment of *Drosophila* S2 cells with 10 mM KMnO₄ for 60 sec, whereas the autoradiograph on the *right* shows results obtained with similarly treated wild-type and R+F 3–4-h-AED *Drosophila* embryos. The graph on the far *right* presents a quantification of the raw Phosphor-Imager data for the S2, wild-type, and R+F samples using the Semi-Automated Footprinting Analysis software package SAFA (Das et al. 2005). The relative sensitivity of each thymine residue is the ratio of the signal for each experimental sample relative to the signal obtained from the 30-sec naked DNA control. A 10-fold or greater increase in KMnO₄ sensitivity is observed for thymine residues at +15, +28, +30, +38, and +50 in both embryo samples, whereas the sensitivity of these residues in S2 cells is nearly indistinguishable from that observed on naked DNA.
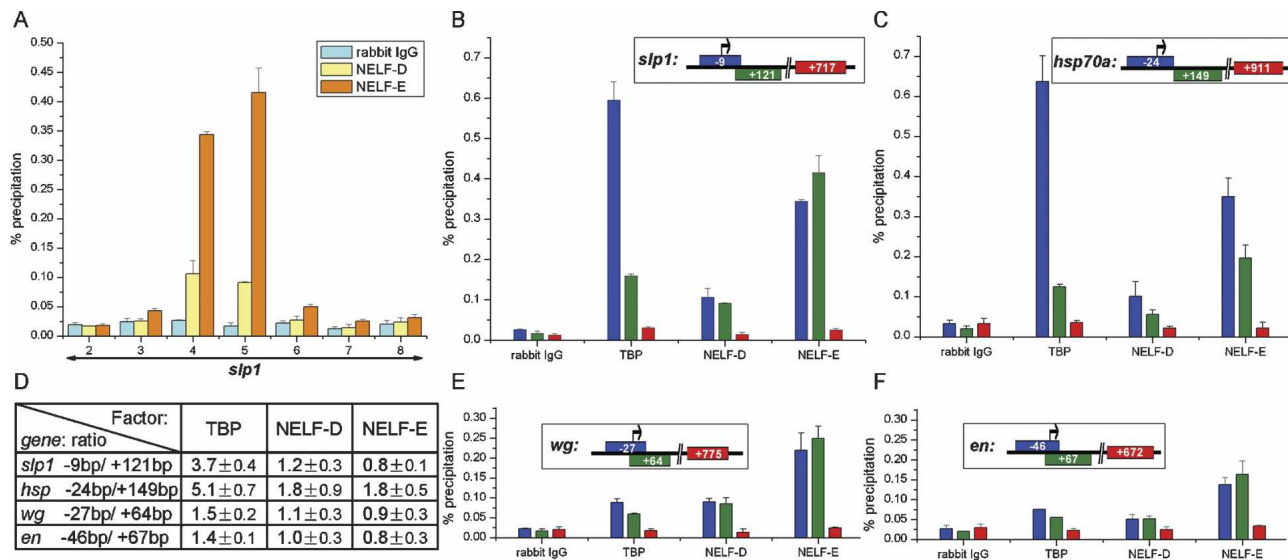
Figure 5. NELF associates with promoter-proximal 5′-UTR regions of segment-polarity genes in the blastoderm embryo. (*A*) ChIP assays on chromatin from wild-type 3–4-h-AED embryos using antisera against the NELF-D (yellow bars) and NELF-E (orange bars) subunits of NELF. The primer pairs used to detect NELF association with different intervals of the *slp1* locus are as described for Figure 2. Background signals obtained using control IgG are shown by cyan bars. (*B*) Comparison of the pattern of TBP, NELF-D, and NELF-E association with intervals of *slp1* detected by primer pairs 4, 5, and 7, centered 9 bp upstream of (blue bars), and 121 bp (green bars) and 717 bp (red bars) downstream from the transcription start site, respectively. (*C*) Pattern of TBP, NELF-D, and NELF-E association with *hsp70a* using a similar representation as in *B*. In this case, the ChIP assays were for intervals centered 24 bp upstream of (blue bars) and 149 bp (green bars) and 911 bp (red bars) downstream from the transcription start site. The preferential association of TBP that is detected with the promoter-proximal versus 5′-UTR *hsp70a* intervals is greater than that observed for *slp1*, presumably due to differences in the exact location of the primer pairs used for these two genes. The slightly stronger signal obtained with both NELF subunits and the *hsp70a* upstream promoter interval indicates NELF association maps closer to –24 bp than to +149 bp, relative to the transcription start site. This is consistent with in vivo data placing the paused Pol II complex 21–35 bp downstream from the *hsp70a* transcription start site (Rasmussen and Lis 1995). (*D*) A table providing the ratios of ChIP signals obtained with promoter-proximal, and 5′-UTR intervals of four different genes (*slp1*, *hsp70a*, *wg*, and *en*) with TBP, NELF-D, and NELF-E antisera. Raw data showing the association of TBP, NELF-D, and NELF-E with different regions of *wg* and *en* are shown in *E* and *F*, respectively. The intervals detected from the *wg* locus centered 27 bp upstream of (blue bars), and 64 bp (green bars) and 775 bp (red bars) downstream from the transcription start site. Similarly for *en*, the PCR-amplified intervals centered 46 bp upstream of (blue bars) and 67 bp (green bars) and 672 bp (red bars) downstream from the transcription start site.

al. 1997). We used this same technique to carry out footprinting studies on the *slp1* promoter region. The results reveal strong hyperreactivity of thymine residues at +15, +28, +30, +38, and +50 downstream from the transcription start site in blastoderm stage embryos (Fig. 4). This interval is similar, though perhaps somewhat larger than the interval detected for *hsp70a*, within which the most prominent increases in reactivity are at residues +22 and +30 (Weber et al. 1997). The pattern of reactivity on *slp1* is extremely similar in both wild-type and *slp1*-repressed embryos, indicating that the hyperreactivity is not due to active transcription of the *slp1* gene. Importantly, this pattern is not observed in nuclei from *Drosophila* tissue culture cells (Fig. 4). Thus, unlike *hsp70a*, the footprint on the *slp1* 5′-UTR is developmentally regulated.

The negative elongation factor NELF is thought to play a key role in establishing the paused Pol II complex on the *hsp70a* promoter (Wu et al. 2003). Indeed, NELF association provides a marker for the paused complex as heat-shock-induced transcriptional elongation involves release of NELF (Wu et al. 2003, 2005). In agreement with the results of our footprinting studies, ChIP experiments reveal the NELF-D and NELF-E subunits are associated with the *slp1* promoter region in chromatin from wild-type embryos (Fig. 5A), but not in chromatin from *Drosophila* tissue culture cells (data not shown). Strong signals are obtained in chromatin from embryos with both the promoter-proximal and 5′-UTR intervals,

whereas background level signals are obtained with other intervals of the *slp1* locus. It is notable that the promoter-proximal signal is less than or equal to the signal detected for the 5′-UTR interval. This pattern of association contrasts that obtained with TBP, which shows a threefold stronger signal with the promoter-proximal primer pair (Fig. 5B,D). Our interpretation of these association patterns is that NELF is bound downstream from the *slp1* transcription start site, presumably as a component of the paused Pol II complex. Consistent with this interpretation, we find a similar differential pattern of TBP and NELF association with promoter-proximal and 5′-UTR intervals of *hsp70a* (Fig. 5C,D). These results strongly suggest that NELF plays a key role in regulating *slp1* elongation in the blastoderm-stage *Drosophila* embryo.

The initial indications that *slp1* expression was regulated at a step downstream from transcription initiation came from ChIP experiments on chromatin from a homogeneous population of embryos that uniformly repress *slp1*. Localized association of NELF in a region downstream from the transcription start site is a hallmark of promoter-proximal pausing. Importantly, this association provides a method for detecting paused Pol II complexes in chromatin from embryos that contain a mixture of cells, some of which are expressing full-length mRNA transcripts. We used ChIP assays to determine whether NELF associates with the promoter re-

gions of *wg* and *en*, two pivotal segment-polarity gene targets of the pair-rule transcription factors. The results reveal specific association of NELF with the promoter-proximal and 5′-UTR regions of both genes in 3–4-h-AED embryos (Fig. 5E,F). Furthermore, the differential association pattern of TBP and NELF with these two intervals indicates that NELF is localized to a region immediately downstream from the initiation sites for both genes (Fig. 5D). These findings indicate that regulation of transcriptional elongation is likely to be central in generating the initial patterns of segment-polarity gene expression in the *Drosophila* embryo.

Regulation of transcriptional elongation has been described for several genes in addition to the *Drosophila* heat-shock genes, including human *c-myc*, *c-myb*, *c-fos*, *junB*, and *p21* (Bender et al. 1987; Pinaud and Mirkovitch 1998; Chen and Sytkowski 2001; Aida et al. 2006; Gomes et al. 2006). A feature shared by these previously characterized examples is rapid induction of gene expression in response to external stimuli. The initial establishment of segment-polarity gene-expression patterns in response to the pair-rule transcription factors occurs within a relatively brief developmental window of ~30 min, spanning the completion of cellularization and the beginning of germ band extension. The temporal advantages offered by regulating these genes at a transcriptional elongation step as compared with chromatin remodeling and/or Pol II initiation complex assembly may be essential for the timely establishment of differing gene expression programs during cell fate specification in the *Drosophila* blastoderm embryo. The observations that Pol II molecules are enriched at the 5′-ends of a number of genes (Gariglio et al. 1981; Rougvie and Lis 1990), coupled with findings that defects in transcriptional elongation factors produce specific developmental defects (Guo et al. 2000; Jennings et al. 2004), strongly suggest that regulation of transcriptional elongation is a hitherto overlooked, but potentially widespread strategy for controlling gene expression during development.

Materials and methods

Drosophila *strains and transgenes*

The *Drosophila* strain *y w[67c23]* that was used to develop different transgenic lines was used as the wild-type control for all in situ hybridization and biochemical experiments. Uniform ectopic expression of different pair-rule genes was obtained using the *NGT* (*nanos-GAL4-tubulin*) maternal GAL4 expression system (Tracey et al. 2000). The genetic cross to express Runt and Opa in embryos mutant for *ftz* was between females homozygous for the *NGT[40]* GAL4-driver and heterozygous for the *ftz[11]* mutation and males homozygous for both the *UAS-runt[15]*and *UAS-opa[14]* transgenes and heterozygous for *ftz[11]*. Ectopic coexpression of Runt and Ftz was obtained by crossing females homozygous for both the *NGT[40]* and *NGT[A]* GAL4 drivers with males homozygous for both the *UAS-runt[15]* and *UAS-ftz[263]* transgenes. These different *NGT*, *UAS-runt*, *UAS-opa*, and *UAS-ftz* transgenes have all been described previously (Swantek and Gergen 2004).

In situ hybridization and RT–PCR

In situ hybridization was carried out as described previously (Swantek and Gergen 2004) using the digoxigenin-labeled riboprobe for *slp1* described in Wheeler et al. (2002). RNA used for PCR after reverse-transcription was isolated from homogenates of ~200 embryos from appropriately staged collections. RNA was extracted using the High Pure RNA isolation Kit (Roche). cDNA was synthesized using the 1st Strand cDNA synthesis Kit (Roche) programmed with 1 ug of RNA. Quantitative PCR was conducted using a Light Cycler (Roche) with a primer pair centered 504 bp downstream from the *slp1* promoter (primer pair 6 in Fig. 2B). The

slp1 RT–PCR signal obtained with different RNA samples was normalized using the RT–PCR signal for *rp49*.

DNase I-hypersensitive site assay

Nuclei isolated from ~250 mg of staged *Drosophila* embryos (Cartwright et al. 1999) were resuspended in DNase I digestion buffer, aliquoted into microfuge tubes, and incubated with 0, 2, 4, or 8 U of DNase I for 3 min on ice. Digestion was stopped by addition of EDTA to 15 mM and SDS to 0.5%. DNA was purified by phenol/chloroform extraction and ethanol precipitation, and resuspended in water. Approximately 5 ug of DNA samples digested with BstXI were run on 1% agarose gels and transferred to nylon membranes for Southern blot hybridization. The *slp1* riboprobe used for these experiments is similar to the one used for in situ hybridization, although it was not subjected to partial hydrolysis prior to use for hybridization. DNA size standards were detected on Southern blots using a digoxigenin-labeled DNA probe.

ChIP

Staged embryo collections were dechorionated, fixed with 2% formaldehyde for 15 min at room temperature, washed, and snap-frozen at –80°C for storage (Orlando et al. 1998). Approximately 100 mg of embryos were homogenized for 1 min in 10 mM EDTA and 50 mM Tris (pH 8.1). After addition of SDS to a final concentration of 1% and incubation on ice for 10 min, glass beads (150–200 µm) were added and the homogenates were sonicated to give sheared chromatin preparations with an average DNA size of 300–400 bp. Chromatin preparations from the equivalent of ~25 mg of embryos were used for each immunoprecipitation using the Chromatin Immunoprecipitation Assay Kit (Upstate Biotechnology) with the following antisera: anti-rabbit IgG (Sigma), anti-mouse IgG (Sigma), anti-mouse IgM (Sigma), anti-Histone H3 (Abcam), anti-acetyl-Histone H3 (Upstate Biotechnology), anti-TBP (Lebedeva et al. 2005), anti-RNA pol II 8WG16 (Covance), anti-RNA pol II H14 (Covance), and anti-NELF-D and anti-NELF-E (Wu et al. 2003). The relative amount of immunoprecipitated DNA was quantified using real-time PCR (Roche Light-Cycler) using primer pairs with a T_m in the range of 59°C–66°C that generate products between 150 and 211 bp. Primer sequences are available on request. The percent precipitation values that are reported were calculated using a dilution series of input chromatin to determine the relative efficiency for each primer pair. PCR was done multiple times on samples from at least two independent immunoprecipitation assays using at least two different chromatin preparations.

Permanganate footprinting

Permanganate footprinting on *Drosophila* S2 cells was performed as described previously using 10 mM $KMnO_4$ for a 1-min incubation (Weber et al. 1997). For embryos, collections of 3–4-h-AED embryos (10 µL, corresponding to ~10^3 embryos or 10^7 genomes) were dechorionated, transferred into 150 µL of ice-cold dissection buffer (130 mM NaCl, 5 mM NaCl, 5 mM KCl, 1.5 mM CaCl2), and briefly homogenized prior to addition of 50 µL of 40 mM $KMnO_4$. Reactions were stopped after 1 min by addition of 200 µL of stop solution (20 mM Tris at pH 7.5, 20 mM NaCl, 40 mM EDTA, 1% SDS, 0.8 M β-mercaptoethanol) to the lysate. Nested primers for detecting cleavage within the *slp1* promoter and 5′-UTR interval by Ligation-Mediated PCR span the interval from 161 to 199 nucleotides (nt) downstream from the transcription start site. The sequences and annealing temperature for PCR are as follows: *slp1* primer-1, 5′-GTTTTGATGGGTTGAGTTG-3′ (51°C); *slp1* primer-2 5′-GGGTTGAGTTGCGGTGT-3′ (55°C); *slp1* primer-3, 5′-GCGGTGTTGATGGGTTTCTT-3′ (58°C).

References

Aida, M., Chen, Y., Nakajima, K., Yamaguchi, Y., Wada, T., and Handa, H. 2006. Transcriptional pausing caused by NELF plays a dual role in

regulating immediate-early expression of the junB gene. *Mol. Cell. Biol.* **26:** 6094–6104.

Akam, M. 1987. The molecular basis for metameric pattern in the *Drosophila* embryo. *Development* **101:** 1–22.

Bender, T.P., Thompson, C.B., and Kuehl, W.M. 1987. Differential expression of c-myb mRNA in murine B lymphomas by a block to transcription elongation. *Science* **237:** 1473–1476.

Boehm, A.K., Saunders, A., Werner, J., and Lis, J.T. 2003. Transcription factor and polymerase recruitment, modification, and movement on dhsp70 in vivo in the minutes following heat shock. *Mol. Cell. Biol.* **23:** 7628–7637.

Cartwright, I.L., Cryderman, D.E., Gilmour, D.S., Pile, L.A., Wallrath, L.L., Weber, J.A., and Elgin, S.C. 1999. Analysis of *Drosophila* chromatin structure in vivo. *Methods Enzymol.* **304:** 462–496.

Chen, C. and Sytkowski, A.J. 2001. Erythropoietin activates two distinct signaling pathways required for the initiation and the elongation of c-myc. *J. Biol. Chem.* **276:** 38518–38526.

Coffman, J.A. 2003. Runx transcription factors and the developmental balance between cell proliferation and differentiation. *Cell Biol. Int.* **27:** 315–324.

Das, R., Laederach, A., Pearlman, S.M., Herschlag, D., and Altman, R.B. 2005. SAFA: Semi-automated footprinting analysis software for high-throughput quantification of nucleic acid footprinting experiments. *RNA* **11:** 344–354.

Gariglio, P., Bellard, M., and Chambon, P. 1981. Clustering of RNA polymerase B molecules in the 5′ moiety of the adult β-globin gene of hen erythrocytes. *Nucleic Acids Res.* **9:** 2589–2598.

Gomes, N.P., Bjerke, G., Llorente, B., Szostek, S.A., Emerson, B.M., and Espinosa, J.M. 2006. Gene-specific requirement for P-TEFb activity and RNA polymerase II phosphorylation within the p53 transcriptional program. *Genes* & *Dev.* **20:** 601–612.

Green, M.R. 2000. TBP-associated factors (TAFIIs): Multiple, selective transcriptional mediators in common complexes. *Trends Biochem. Sci.* **25:** 59–63.

Guo, S., Yamaguchi, Y., Schilbach, S., Wada, T., Lee, J., Goddard, A., French, D., Handa, H., and Rosenthal, A. 2000. A regulator of transcriptional elongation controls vertebrate neuronal development. *Nature* **408:** 366–369.

Howard, K. 1990. The blastoderm prepattern. *Semin. Cell Biol.* **1:** 161–172.

Ingham, P.W. 1988. The molecular genetics of embryonic pattern formation in *Drosophila*. *Nature* **335:** 25–34.

Jennings, B.H., Shah, S., Yamaguchi, Y., Seki, M., Phillips, R.G., Handa, H., and Ish-Horowicz, D. 2004. Locus-specific requirements for Spt5 in transcriptional activation and repression in *Drosophila*. *Curr. Biol.* **14:** 1680–1684.

Jones, J.C., Phatnani, H.P., Haystead, T.A., MacDonald, J.A., Alam, S.M., and Greenleaf, A.L. 2004. C-terminal repeat domain kinase I phosphorylates Ser2 and Ser5 of RNA polymerase II C-terminal domain repeats. *J. Biol. Chem.* **279:** 24957–24964.

Komarnitsky, P., Cho, E.J., and Buratowski, S. 2000. Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. *Genes* & *Dev.* **14:** 2452–2460.

Lebedeva, L.A., Nabirochkina, E.N., Kurshakova, M.M., Robert, F., Krasnov, A.N., Evgen'ev, M.B., Kadonaga, J.T., Georgieva, S.G., and Tora, L. 2005. Occupancy of the *Drosophila* hsp70 promoter by a subset of basal transcription factors diminishes upon transcriptional activation. *Proc. Natl. Acad. Sci.* **102:** 18087–18092.

Levanon, D. and Groner, Y. 2004. Structure and regulated expression of mammalian RUNX genes. *Oncogene* **23:** 4211–4219.

Morris, D.P., Michelotti, G.A., and Schwinn, D.A. 2005. Evidence that phosphorylation of the RNA polymerase II carboxyl-terminal repeats is similar in yeast and humans. *J. Biol. Chem.* **280:** 31368–31377.

Orlando, V., Jane, E.P., Chinwalla, V., Harte, P.J., and Paro, R. 1998. Binding of trithorax and Polycomb proteins to the bithorax complex: Dynamic changes during early *Drosophila* embryogenesis. *EMBO J.* **17:** 5141–5150.

Patturajan, M., Conrad, N.K., Bregman, D.B., and Corden, J.L. 1999. Yeast carboxyl-terminal domain kinase I positively and negatively regulates RNA polymerase II carboxyl-terminal domain phosphorylation. *J. Biol. Chem.* **274:** 27823–27828.

Pinaud, S. and Mirkovitch, J. 1998. Regulation of c-fos expression by RNA polymerase elongation competence. *J. Mol. Biol.* **280:** 785–798.

Rasmussen, E.B. and Lis, J.T. 1995. Short transcripts of the ternary complex provide insight into RNA polymerase II elongational pausing. *J. Mol. Biol.* **252:** 522–535.

Roth, S.Y., Denu, J.M., and Allis, C.D. 2001. Histone acetyltransferases. *Annu. Rev. Biochem.* **70:** 81–120.

Rougvie, A.E. and Lis, J.T. 1988. The RNA polymerase II molecule at the 5′ end of the uninduced hsp70 gene of *D. melanogaster* is transcriptionally engaged. *Cell* **54:** 795–804.

Rougvie, A.E. and Lis, J.T. 1990. Postinitiation transcriptional control in *Drosophila melanogaster*. *Mol. Cell. Biol.* **10:** 6041–6045.

Schubeler, D., MacAlpine, D.M., Scalzo, D., Wirbelauer, C., Kooperberg, C., van Leeuwen, F., Gottschling, D.E., O'Neill, L.P., Turner, B.M., Delrow, J., et al. 2004. The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. *Genes* & *Dev.* **18:** 1263–1271.

Sims III, R.J., Belotserkovskaya, R., and Reinberg, D. 2004. Elongation by RNA polymerase II: The short and long of it. *Genes* & *Dev.* **18:** 2437–2468.

Swantek, D. and Gergen, J.P. 2004. Ftz modulates Runt-dependent activation and repression of segment-polarity gene transcription. *Development* **131:** 2281–2290.

Tracey Jr., W.D., Ning, X., Klingler, M., Kramer, S.G., and Gergen, J.P. 2000. Quantitative analysis of gene function in the *Drosophila* embryo. *Genetics* **154:** 273–284.

Weber, J.A., Taxman, D.J., Lu, Q., and Gilmour, D.S. 1997. Molecular architecture of the hsp70 promoter after deletion of the TATA box or the upstream regulation region. *Mol. Cell. Biol.* **17:** 3799–3808.

Wheeler, J.C., VanderZwan, C., Xu, X., Swantek, D., Tracey, W.D., and Gergen, J.P. 2002. Distinct in vivo requirements for establishment versus maintenance of transcriptional repression. *Nat. Genet.* **32:** 206–210.

Wu, C.H., Yamaguchi, Y., Benjamin, L.R., Horvat-Gordon, M., Washinsky, J., Enerly, E., Larsson, J., Lambertsson, A., Handa, H., and Gilmour, D. 2003. NELF and DSIF cause promoter proximal pausing on the hsp70 promoter in *Drosophila*. *Genes* & *Dev.* **17:** 1402–1414.

Wu, C.H., Lee, C., Fan, R., Smith, M.J., Yamaguchi, Y., Handa, H., and Gilmour, D.S. 2005. Molecular characterization of *Drosophila* NELF. *Nucleic Acids Res.* **33:** 1269–1279.