

Stopping transcription in its tracks

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A new study of *Drosophila melanogaster* demonstrates that the establishment and maintenance of transcriptional repression are distinct processes that can be genetically dissected.

The generation of the anteroposterior segmental pattern of the *D. melanogaster* embryo has been the subject of intense genetic analysis for more than 20 years. This model system has revealed many key molecules and mechanisms involved in development, gene regulation and cell–cell signaling in all animals. Indeed, some might argue that, after such long and rigorous investigation, there is little more we can learn from studying segmentation in the fruit fly. But an elegant genetic study presented in the accompanying paper by John Wheeler and colleagues¹ has produced some new and perhaps surprising insights into the different mechanisms used to achieve transcriptional repression during development. The authors investigated how Runt, the founder member of the Runt-domain (or Runx) family of transcription factors, can mediate transcriptional repression during segmentation in *D. melanogaster*. Their results indicate that this repression is a two-step process and that the establishment and maintenance of repression are distinct.

Runt-domain proteins

Runt-domain proteins are important in many developmental processes, including hematopoiesis, bone development and development of the gastrointestinal tract. Mutations in the corresponding genes have been implicated in cancers and bone disease^{2,3}. Indeed, *RUNX1* has long been recognized as an important translocation breakpoint in human leukemias, and *RUNX3* has recently been touted as a candidate tumor-suppressor gene in gastric cancer⁴. Naturally there is widespread interest in understanding how these proteins work *in vivo*.

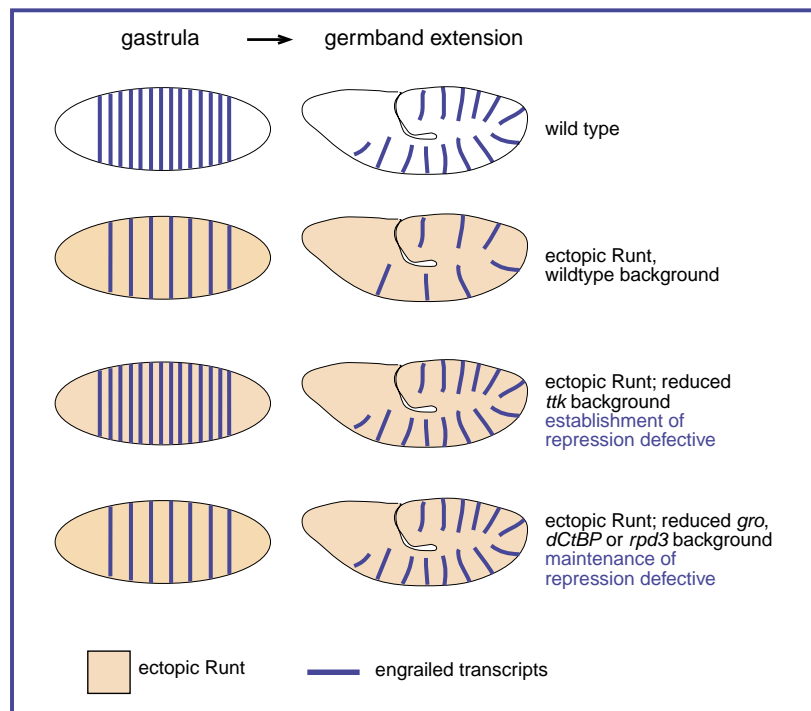
The Runt protein in *D. melanogaster* is encoded by a pair-rule gene and acts to establish segmental pattern in the early embryo⁵. It is expressed in seven stripes across the embryo and regulates expression of a number of other segmentation genes, including *hairy*, *even-skipped (eve)*, *fushi tarazu (ftz)* and *engrailed (en)*. It also acts during sex determination and devel-

opment of the nervous system. Another Runt-domain protein in the fly, Lozenge, is important for cell-fate decisions during eye development and hematopoiesis⁵. All of these proteins have a Runt domain, a 128-amino-acid motif required for DNA binding and for dimerization with an unrelated partner protein that modulates DNA-binding activity⁶. In mammals, the known partner protein is core-binding factor β (CBF- β). In the fly, there are two known partner proteins that are orthologous to CBF- β : Brother (Bro) and Big Brother (Bgb).

Depending on the target gene and developmental context, Runt-domain proteins act as either transcriptional acti-

vators or repressors⁶. As repressors, the Runt-domain proteins can interact with the Groucho family co-repressors through a conserved carboxy-terminal pentapeptide motif, VWRPY. In some situations, however, Runt-domain proteins seem to repress transcription independently of Groucho, through interactions with unknown factors⁷.

Ectopic expression of Runt in the early stages of *D. melanogaster* development blocks transcription of *en* in the odd-numbered stripes and is lethal to the embryo. The authors took advantage of this to carry out a genetic screen for factors that potentiate Runt activity. They looked for gene products placed into the



Staying power. The timing of recovery of *en* expression in the presence of ectopic Runt differs depending on the genetic background. Wheeler *et al.*¹ show that in a wildtype background, ectopic Runt represses expression of the odd-numbered stripes of *en* at both the gastrula and germband extension phases of development. When the dose of *ttk* is reduced, all 14 *en* stripes are expressed at the gastrula stage, indicating that Runt-mediated repression has completely failed. However, when the dose of *gro*, *dCtBP* or *tpd3* is reduced, ectopic Runt represses *en* expression in the gastrula stage but not at germband extension when all 14 stripes of *en* are observed. This indicates that repression of *en* was initiated but could not be maintained and that there are establishment and maintenance phases in Runt-mediated repression.

egg by the mother that are needed for the activity of ectopic Runt. They identified four loci that, when present in only one gene dose, relieve Runt repression of *en* transcription in the odd-numbered stripes and thus rescue the embryo.

Two of these genes encode proteins previously shown to be involved in transcriptional repression as non-DNA-binding co-factors: dCtBP⁸ and Groucho⁹. The other two identified were *tramtrack* (*ttk*)¹⁰ and the locus encoding the histone deacetylase Rpd3 (ref. 11), which was previously implicated in transcriptional repression. The gene *ttk* encodes two isoforms (of 69 kD and 88 kD) that act as DNA-binding transcriptional repressors. The two proteins are generated by alternative splicing and share a common amino terminus (containing a BTB/POZ domain), but their C-terminal portions contain differing zinc-finger domains with distinct DNA-binding specificities. The genetic analysis presented by Wheeler *et al.*¹ indicates that it is the 88-kD isoform of Tramtrack that is predominantly required by Runt for repression of *en* transcription.

A genetic distinction

The key observation made by Wheeler *et al.*¹ is that the loss of *en* expression caused by ectopic Runt is only temporary, and that the timing of recovery differs depending on genetic background (see figure). Crucially, when the dose of *ttk* is reduced, *en* transcription is restored at the gastrula stage. By contrast, *en* expression returns about 30 minutes later, during germband extension when the dose of Groucho, dCtBP or RPD3 is reduced. Furthermore, ectopic expression of Runt protein lacking either the Groucho-interaction domain or the DNA-binding motif represses *en* transcription at the gastrula stage, but this repression fails by the germband-extension stage and *en* expression recovers. Thus, the authors propose a model in which Runt represses *en* transcription in two phases: an establishment

phase that requires Tramtrack but does not require high-affinity DNA binding by Runt, and a later maintenance phase requiring Groucho, dCtBP and Rpd3, and DNA-binding by Runt.

Earlier models had suggested that Runt-domain proteins bind DNA directly in collaboration with CBF- β and co-repressor proteins (Groucho, for example) to mediate repression. As such, the discovery of the requirement for Tramtrack in Runt-mediated repression must have come as quite a surprise. A previous study¹⁰ shows that the 88-kD isoform of Tramtrack binds DNA sequences in the *en* regulatory region. Wheeler *et al.*¹ propose a reasonable, if speculative, model whereby Tramtrack binds DNA and recruits Runt to the *en* regulatory region to establish transcriptional repression, possibly by interacting directly with components of the basal transcription machinery. To maintain repression, Runt subsequently binds DNA directly and recruits dCtBP and Groucho. In turn, Groucho recruits Rpd3 to deacetylate histones and change chromatin conformation to a transcriptionally inactive state.

Maintenance of repression

Other results¹¹ concerning the role of Rpd3 in segmentation have been somewhat confusing. Embryos with reduced maternal Rpd3 have a pair-rule segmentation phenotype. It has been suggested that Rpd3 interacts with Groucho and other transcriptional regulators, but none of these interactions adequately explains the mutant phenotype. In addition, the expression of several pair-rule genes seems normal, as does the initial expression of *en* expression. The study by Wheeler *et al.*¹ also demonstrates that, like Runt, Eve and Sloppy-paired 2 (proteins unrelated to Runt or each other) can establish repression of target genes in embryos containing severely reduced Rpd3 activity. Thus it seems that chromatin modification by Rpd3 is not required to repress transcription *per se*,

but is needed to prevent reactivation if factors that promote 'active' chromatin persist.

Perhaps the establishment phase of repression reflects the molecular process that initially blocks formation of an active pre-initiation complex to halt transcription. Establishment of repression could be the process by which sequence-specific DNA-binding factors are recruited to the appropriate regulatory region with co-factors and inhibit the active transcription machinery directly. But without more chemically stable modifications of the promoter region—histone deacetylation and resultant changes in chromatin structure, for example—competing activating factors can ultimately overcome this block to re-establish transcription.

A precedent for the separation of establishment and maintenance of transcriptional repression comes from the *polycomb* group (*PcG*) genes¹². For example, the patterns of *Hox* transcription set up by transiently expressed factors during development are subsequently maintained in a very stable and heritable manner by chromatin modifications mediated by the *PcG* proteins. Additional studies of other transcriptional repressors in other suitable systems should show just how universal is the model for separable phases of establishment and maintenance of transcriptional repression. □

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