Chapter 8. Developmental constraints in *Drosophila*, mitotic domains and activation of the zygotic genome.

The embryonic and larval stages of the fly develop rapidly as the larvae compete with yeasts and bacteria for the nutrients in rotting fruit. This extreme time constraint canalises developmental mechanisms that are common with other multicellular organisms. The first rapid syncytial divisions take about eight minutes at the centre of the egg. After the eight division, the majority of nuclei migrate outwards to arrive at the cortex during cycle 10; leaving behind a few central (yolk) nuclei with limited transcriptional activity [1] [2]. Between cycles 10 and 13, nuclear divisions are initiated near the embryonic poles and spread inwards around the embryonic cortex, taking about half a minute to reach the equatorial mid-point. Cycle 10 takes 8.8 minutes, slowing to 17 minutes by cycle 13. General activation of the zygotic genome takes place during the 14th cycle; as cortical nuclei become enclosed by membrane in-growth [3] [4]. At this stage, the division cycle slows to about 65 minutes; late-replicating, heterochromatic DNA domains are formed, together with stable histone modifications, and the release of promoter-paused RNA PolymeraseII (PolII) complexes [5] [6] [7] [8]. Following this "mid-blastoderm transition", metachronal waves spread across discrete mitotic domains, with spindle orientations aligned between adjacent cells, and with respect to the embryonic axes [3]. The slow progression of metachronal waves across these mitotic domains results in cell-cycle delays of up to two hours for the last-dividing cells [3] [4]. Critically, the aligned spindle polarities are scalar, having direction but not magnitude. However, the differing cell-cycle durations represent a second scalar component. In principle, therefore, the mitotic domains could transmit a textured pre-pattern: converting maternally inherited morphogen gradients into the framework for regulating zygotic gene expression.

The first indication of an embryonic midline is a ventral furrow of apically constricted cells towards the end of cycle 14. A dorsal cephalic fold is initiated at the same stage, followed by anterior and posterior folds along the trunk [3]. As gastrulation begins, the dorsal folds extend ventrally around the D/V axis, on the left (L) and right (R) embryonic flanks. The key genes regulating D/V axis formation in *Drosophila* were identified by Nusslein-Volhard, Wieschaus and co-workers [9] [10] [11] [12] [13]. The ventral mid-line cells form an axis of mirror symmetry (AMS) in the epithelial plane, with an apico-basal rotation around their radial axis from the egg centre (see below, **23)**. These morphogenetic movements are regulated by an extracellular gradient of Tl around the D/V (L/R) axis, which is transduced to a nuclear gradient of Dorsal (Dl) activity [14]. Dl is homologous to the mammalian NF-κB transcription factor (TF) and regulates a set of genes including *decapentaplegic (dpp*, the TGF-β receptor), *twist* (*twi*) and *snail* (*sna*). In *twi* and *sna* mutants, the embryonic ventral furrow and mesodermal lineages fail to form. These defects are associated with delayed formation of the mesoderm-specific mitotic domains in *sna* and their ventral shift in *twi* [15]. Constitutive activation of the Tl pathway blocks formation of the ventral furrow, the cephalic, anterior and posterior folds, and the segmentation cascade; in addition, the *sna* domain is expanded and all embryonic cells adopt a mesodermal fate [16]. By contrast, mutants that block the Tl signalling cascade form disorganised epithelial tubes. These mutant defects can be rescued by injection of wild-type perivitelline fluid, which resets the embryonic polar co-ordinates. As this happens, the embryonic axial system is rotated, dependent on the site at which the perivitelline fluid is injected [17]. Taken together, these results imply that the Toll signalling cascade sets the orthogonal embryonic axes, in conjunction with surface boundary effects around the egg cortex.

The long (A/P) embryonic axis is specified by gradients of Caudal (Cad) and Bicoid

(Bic). Embryos deficient for *bic* develop two abdomens without a head, while injection of *bic* RNA into the syncytial blastoderm induces head structures at any point along the A/P axis, with altered segmental progression [18]. However, while regional fate is altered, the segmental boundaries remain orthogonal to the D/V axis: by this criterion the embryonic coordinates are not rotated. Each step of the segmentation cascade is regulated by inhibitory feedback loops around both the D/V and A/P embryonic axes. The initial asymmetry is provided via maternal "coordinate" genes, which govern the distribution of "gap genes", reviewed in [19]. In turn, gap genes, regulate the expression of zygotic "pair-rule" genes in alternate segments. Below the pair-rule genes, "segment-polarity" genes define individual segmental repeats. Most genes in this cascade encode TFs, or the morphogens and growth factors (GFs) that regulate their expression. The individual gene products form antagonistic pairs, such that the initial gap gene bias is sharpened via negative feedback between of pair-rule functions. In particular, the position of alternate stripes is determined by the expression of *fushi-tarazu (ftz)* and *even-skipped (eve)* [20]. The relative levels of *ftz* and *eve* expression determine the width of alternate stripes, with abnormally thin stripes being eliminated by cellular delamination and apoptosis [21]. Taken together these results suggest that the role of the segmentation cascade may be to digitise the opposing Cad and Bic gradients, so that each ring of cells around the embryonic circumference is assigned a unique fate. The cascade is stabilised and refined by nested, regulatory feed-back loops. Within this network of interactions, the differential fate of individual segments is allocated via the homeotic (Hox) genes of the *Antennapedia* and *bithorax* complexes (Antp-C and Bx-C) [22 23]. Surprisingly, the domains of action of these homeotic genes define "parasegmental" units [24], which are 90^{o} out of phase with the segmental boundaries. Thus, the segmentation cascade resembles an oscillating system with morphogenetic twin-fields as a harmonic standing wave, with an overlaid A > P bias in parasegmental fate. The pitch of the standing wave may alter during normal development, with sub-division of morphogenetic twin-fields.

The simplest mechanism for keying expression of the segmentation cascade to the cell-cycle may be via intron delay [25]. The rapid embryonic divisions allow only short transcripts to be completed within a single division cycle, with a transcription rate of about 1.4 kb per minute [26 27]. This transcriptional delay has global effects on zygotic gene expression [28 29]. Indeed, many transcription units (TUs) have cryptic non-coding 5' exons that allow differential regulation from alternative promotors [30]. In addition the *Drosophila* genome includes several megabase-length TUs encoding the motor proteins: Myo81F (1.97 mb) and the Dyneins Kl-3 (2.3 mb), Kl-5 (1.2 mb), FlyBase, [31]. Additional cell-cycle dependent regulatory mechanisms may be linked to 5' promoter pausing, splicing-delay, cytoplasmic/nucleolar shuttling, 3' mRNA processing and degradation rates [32 33 34 35 36 37]. In contrast to the fruit-fly, vertebrate segments are recruited from P > A with oscillating expression of segmentation genes within each myotome [38]. In the zebrafish and mouse, segmentation may be regulated by transcriptional delay [39 40 41]. In particular, the expression of murine Hes7 travels in progressive waves along the dorsal midline, with a period of about 2 hours. Transcription of the endogenous *Hes7* gene takes about 19 minutes, but expression of an intron-less *Hes7* transgene oscillates abnormally and gives severe segmentation defects [42]. In the mouse it is probably the intron-splicing rate, rather than uniform progression of the PolII transcription complex, which accounts for the major delay (Kageyama, personal communication). Splicing-delay also regulates gene expression in mouse fibroblasts *in vitro* and during *Drosophila* embryogenesis [43 44].

Summary:
The initial syncytial divisions of *Drosophila* eggs are randomly oriented. As nuclei migrate outwards from the egg centre their spindle poles align to the curved surface of

the embryo. Mitotic waves initiated near the embryonic poles spread in towards the A/P equatorial midline during the next 3 nuclear divisions. The zygotic genome is fully activated during cycle 14, as the membrane downgrowth partitions the individual nuclei of the cellular blastoderm. At this stage, the division cycle slows down and stable chromatin modifications are imposed. Maternally derived factors set the initial embryonic axes and expression of the zygotic transcriptome. Following cellularisation, metachronal waves may transmit vectorial information; with associated cell-shape changes, oriented mitotic spindles and differential cell-cycle durations. A nested cascade of regulatory feedback loops sets the embryonic axial system, with gene expression keyed to the cell cycle. Vertebrate segmentation is similarly delimited by cell-cycle dependent mechanisms along a D (L/R) midline.

References:

1. Foe, V. E. & Alberts, B. M. Studies of nuclear and cytoplasmic behaviour during the five mitotic cycles that precede gastrulation in Drosophila embryogenesis. *J. Cell Sci.* 61, 31–70 (1983).
2. Campos-Ortega, J. A. & Hartenstein, V. *The Embryonic Development of Drosophila Melanogaster.* (1985).
3. Foe, V. E. Mitotic domains reveal early commitment of cells in Drosophila embryos. *Development* 107, 1–22 (1989).
4. Foe, V. E., Odell, G. M. & Edgar, B. A. Mitosis and morphogenesis in the Drosophila embryo: Point and counterpoint. *Dev. Drosoph. Melanogaster* 149–300 (1993).
5. Foe, V. E. & Alberts, B. M. Studies of nuclear and cytoplasmic behaviour during the five mitotic cycles that precede gastrulation in Drosophila embryogenesis. *J. Cell Sci.* 61, 31–70 (1983).
6. Seller, C. A., Cho, C.-Y. & O'Farrell, P. H. Rapid embryonic cell cycles defer the establishment of heterochromatin by Eggless/SetDB1 in Drosophila. *bioRxiv* 450155 (2018) doi:10.1101/450155.
7. Chen, K. *et al.* A global change in RNA polymerase II pausing during the Drosophila midblastula transition. *eLife* 2, e00861 (2013).
8. Li, X.-Y., Harrison, M. M., Villalta, J. E., Kaplan, T. & Eisen, M. B. Establishment of regions of genomic activity during the Drosophila maternal to zygotic transition. *eLife* 3, e03737 (2014).
9. Nusslein-Volhard, C. & Wieschaus, E. Mutations affecting segment number and polarity in Drosophila. *Nature* 287, 795–801 (1980).
10. Nusslein-Volhard, C., Wieschaus, E. & Kluding, H. Mutations affecting the pattern of the larval cuticle in Drosophila melanogaster. *Rouxs Arch. Dev. Biol.* 193, 267–282 (1984).
11. Wieschaus, E., Nusslein-Volhard, C. & Jurgens, G. Mutations affecting the pattern of the larval cuticle in Drosophila melanogaster. *Rouxs Arch. Dev. Biol.* 193, 296–307 (1984).
12. Jurgens, G., Wieschaus, E., Nusslein-Volhard, C. & Kluding, H. Mutations affecting the pattern of the larval cuticle in Drosophila melanogaster. *Rouxs Arch. Dev. Biol.* 193, 283–295 (1984).
13. Anderson, K. V., Jurgens, G. & Nusslein-Volhard, C. Establishment of dorsal-ventral polarity in the Drosophila embryo. Genetic studies on the role of the Toll gene product. *Cell* 42, 779–789 (1985).

14. Anderson, K. V. & Nusslein-Volhard, C. Genetic analysis of the dorso-ventral embryonic pattern in Drosophila. *Pattern Form. Primer Dev. Biol.* 269–289 (1984).
15. Arora, K. & Nusslein-Volhard, C. Altered mitotic domains reveal fate map changes in Drosophila embryos mutant for zygotic dorsoventral patterning genes. *Development* 114, 1003–1024 (1992).
16. Ligoxygakis, P., Roth, S. & Reichhart, J-M. A serpin regulates dorsal-ventral axis formation in the Drosophila embryo. *Curr. Biol.* 13, 2097–2102 (2003).
17. Stein D, Roth S, Vogelsang E, & Nusslein-Volhard C. The polarity of the dorsoventral axis in the drosophila embryo is defined by an extracellular signal. *Cell* 65, 725–735 (1991).
18. Driever, W., Siegel, V. & Nusslein-Volhard, C. Autonomous determination of anterior structures in the early Drosophila embryo by the bicoid morphogen. *Development* 109, 811 (1990).
19. Gilbert, S. F. *Developmental Biology*. (Sinauer Associates, 2006).
20. Lawrence, P. A., Johnston, P., MacDonald, P. & Struhl, G. Borders of parasegments in Drosophila embryos are delimited by the fushi tarazu and even-skipped genes. *Nature* 328, 440–442 (1987).
21. Hughes, S. C. & Krause, H. M. Establishment and maintenance of parasegmental compartments. *Development* 128, 1109–1118 (2001).
22. Li, X. & McGinnis, W. Activity regulation of Hox proteins, a mechanism for altering functional specificity in development and evolution. *Proc. Natl. Acad. Sci. U. S. A.* 96, 6802–6807 (1999).
23. Lewis, E. B. Regulation of the genes of the Bithorax complex in Drosophila. *Cold Spring Harb. Symp. Quant. Biol.* 50, 155–164 (1985).
24. Martinez Arias, A. & Lawrence, P. A. Parasegments and compartments in the Drosophila embryo. *Nature* 313, 639–642 (1985).
25. Gubb, D. Intron-delay and the precision of expression of homoetic gene products in Drosophila. *Dev. Genet.* 7, 119–131 (1986).
26. Shermoen, A. W. & O'Farrell, P. H. Progression of the cell cycle through mitosis leads to abortion of nascent transcripts. *Cell* 67, 303–310 (1991).
27. Thummel, C. S. Mechanisms of transcriptional timing in Drosophila. *Science* 255, 39–40 (1992).
28. Manak, J. R. *et al.* Biological function of unannotated transcription during the early development of Drosophila melanogaster. *Nat. Genet.* 38, 1151–1158 (2006).
29. Artieri, C. G. & Fraser, H. B. Transcript length mediates developmental timing of gene expression across Drosophila. *Mol. Biol. Evol.* 31, 2879–2889 (2014).
30. Manak, J. R. *et al.* Biological function of unannotated transcription during the early development of Drosophila melanogaster. *Nat. Genet.* 38, 1151–1158 (2006).
31. Fingerhut, J. M., Moran, J. V. & Yamashita, Y. M. Satellite DNA-containing gigantic introns in a unique gene expression program during Drosophila spermatogenesis. *PLoS Genet.* 15, e1008028 (2019).
32. DeLotto, R., DeLotto, Y., Steward, R. & Lippincott-Schwartz, J. Nucleocytoplasmic shuttling mediates the dynamic maintenance of nuclear Dorsal levels during Drosophila embryogenesis. *Development* 134, 4233 (2007).
33. Chanet, S. & Schweisguth, F. Regulation of epithelial polarity by the E3 ubiquitin ligase Neuralized and the Bearded inhibitors in Drosophila. *Nat. Cell Biol.* 14, 467–476 (2012).
34. Rodrigues-Campos, M. & Thompson, B. J. The ubiquitin ligase FbxL7 regulates the Dachsous-Fat-Dachs system in Drosophila. *Development* 141, 4098–4103 (2014).

35. Audas, T. E., Jacob, M. D. & Lee, S. Immobilization of proteins in the nucleolus by ribosomal intergenic spacer noncoding RNA. *Mol. Cell* 45, 147–157 (2012).
36. Saunders, A., Core, L. J., Sutcliffe, C., Lis, J. T. & Ashe, H. L. Extensive polymerase pausing during Drosophila axis patterning enables high-level and pliable transcription. *Genes Dev.* 27, 1146–1158 (2013).
37. Tsai, S.-Y., Chang, Y.-L., Swamy, K. B. S., Chiang, R.-L. & Huang, D.-H. GAGA factor, a positive regulator of global gene expression, modulates transcriptional pausing and organization of upstream nucleosomes. *Epigenetics Chromatin* 9, 32–32 (2016).
38. Palmieri, R. M., Ingersoll, C. D. & Hoffman, M. A. The Hoffmann reflex: methodologic considerations and applications for use in sports medicine and athletic training research. *J. Athl. Train.* 39, 268–277 (2004).
39. Lewis, J. Autoinhibition with transcriptional delay: a simple mechanism for the zebrafish somitogenesis oscillator. *Curr. Biol. CB* 13, 1398–1408 (2003).
40. Bessho, Y., Hirata, H., Masamizu, Y. & Kageyama, R. Periodic repression by the bHLH factor Hes7 is an essential mechanism for the somite segmentation clock. *Genes Dev.* 17, 1451–1456 (2003).
41. Swinburne, I. A. & Silver, P. A. Intron delays and transcriptional timing during development. *Dev. Cell* 14, 324–330 (2008).
42. Takashima, Y. *et al.* Intronic delay is essential for oscillatory expression in the segmentation clock. *Proceedings of the National Academy of Sciences of the United States of America* 3300 (2011).
43. Hao, S. & Baltimore, D. RNA splicing regulates the temporal order of TNF-induced gene expression. *Proc. Natl. Acad. Sci. U. S. A.* 110, 11934–11939 (2013).
44. Guilgur, L. G. *et al.* Requirement for highly efficient pre-mRNA splicing during Drosophila early embryonic development. *eLife* 3, e02181 (2014).