Chapter 24. The Hox-C regulatory hubs and downstream morphogenetic interactions.

General transcription of the zygotic genome is activated during the mid-blastoderm transition; with the Hox genes expressed during gastrulation and germ band extension. A single set of Hox genes is separated into the Antennapedia (Ant-C) and Bithorax (Bx-C) complexes in *Drosophila*. Parasegmental identity is co-linear with the chromosomal order of the Hox genes [1 2 3 4 5], with alternative (orthosegmental) D/V fates. The prescient genetic studies of E. B. Lewis established that Bx-C mutants are sensitive to chromosomal position effects acting at the boundaries between open and condensed chromatin domains; and that transvection between paired chromosomal bivalents regulates key morphogenetic functions [1 3 6 7 8 9 10]. Chromosomal compaction is regulated by Polycomb (Pc), with the Hox complexes remaining densely packed (and late-replicating) during the mid-blastoderm transition, data of [11]. In general, extended TUs outside the Hox complexes while may show Pc-repressed exons, but their intronic segments, and any nested transcripts, tend not to be, data of [12] displayed on FlyBase, JBrowse (https://flybase.org/) [13] (Fig. 31), see also [14].

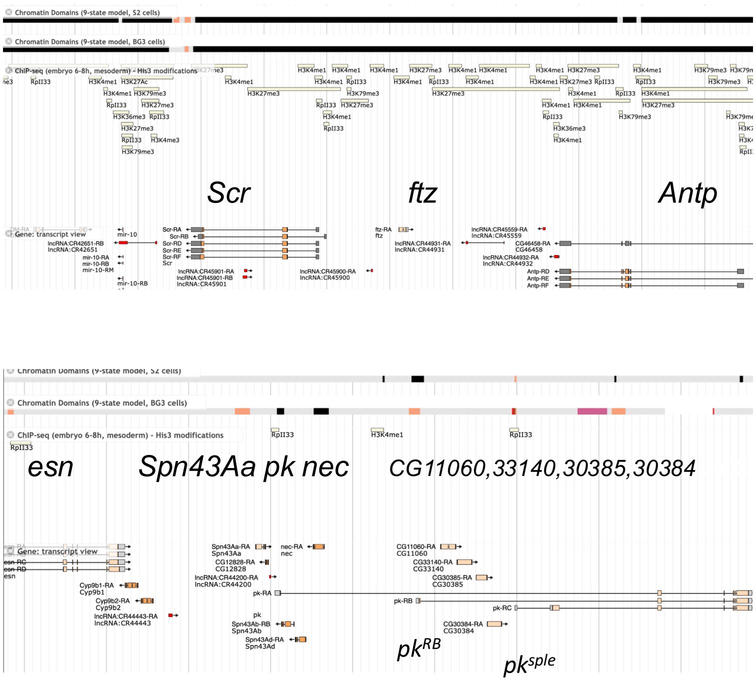

Fig. 31. Hox complexes genes are late replicating, hypermethylated and PC repressed. A. The *scr*, *ftz* and *Antp* TUs. However, extended genes outside the Hox complexes may have Pc-repressed exons, but their intronic segments, and any nested transcripts, tend not to be. For example: **B.** The coding exons of *pk* are repressed during the mid-blastoderm transition. However, the *pk* intronic segments are hypomethylated as are its nested, compact TUs. From FlyBase, JBrowse view.

The Pc group proteins regulate chromatin condensation via methylation of Histone3 lysine residues: H3-K27^{me3} and H3-K9$^{me2/3}$ [15 16 17 18]. These covalent modifications are antagonised by the Histone methyl transferases *trithorax* (*trx*) and *trx-like*, which control Pc-silencing [19]. Trx binds to Polycomb Response Elements (PREs) within the Hox gene complexes, as well as PRE sites in *en* and *inv*, the *Iroquois-complex* (Iro-C) and *Cyclin A*; with about 500 putative PRE sites distributed across the genome [18, 20 21 22]. Within the *Bx-C*, differential H3-K27 modifications are co-linear with parasegmental boundaries [16]. Similarly, the temporal progression of active and silent chromatin domains in the human *Hox-C* complex is correlated with H3-K27 modifications [23 24].

The anterior regions of *Drosophila* are specified by the *Ant-C* genes: *labial* > *proboscipedia* > *Deformed* > *Sex combs reduced* > *Antennapedia* (*lab*, *pb*, *Dfd*, *Scr*, *Antp*); while thoracic and abdominal fates are regulated via the *Bx-C*: *Ultrabithorax* > *abdominal-A* > *Abdominal-B* (*Ubx*, *abdA*, *AbdB*). A unique Hox transcript is not deployed in each of the thoracic and abdominal parasegments, where quantitative differences in expression allocate successive fates [25]. Instead, thoracic identities are specified by *Ubx*, with minor contributions from *Antp, abd-A* and *Abd-B*; while abdominal fates are dependent on *abd-A*, with progressively less *Ubx* and more *Abd-B* from A > P. Sexually dimorphic terminal fates are allocated by alternatively-spliced *abd-A* and *Abd-B* transcripts, as the migrating germ-line stem cells populate the genital ridge[26]. The Hox complexes register the asymmetry of zygotic gap gene expression with *Antp* activated via *hb*; while *abdA* and *AbdB* respond to the overlapping domains of *hb*, *Kruppel* (*Kr*), *tailless* (*tll*) and *knirps* (*kni*)[26]. In particular, the *tll* steroid receptor binds a PolII-specific hormone response element (HRE), initially at both embryonic poles, but later restricted to the P pole and the AD midline [27]. Meanwhile, the Kr transcriptional suppressor is expressed in a broad band around the thoracic midline, with both the Kr and Tll TFs binding to the Hr78 hormone receptor [28]. In principle, these protein interactions are consistent with the embryonic A/P progression being co-ordinately regulated by hormonal signal receptors and transcriptional repression. In the trunk region, *Antp* is suppressed by each of the Bx-C genes, *Ubx*, *abd-A* and *Abd-B* [29]. Lack of *Abd-B* transforms the terminal abdominal segments to a more anterior fate; while somatic clones in the genital discs may activate expression of the *Distalless* (*Dll*) Hox co-factor, giving homoeotic transformation to terminal antennal, or leg segments [30].

The Hox TUs vary in length between 10.6 kb (*Dfd*) to 108.9 kb (*Ubx*); with additional compact homeobox transcripts in the *Ant-C*: *ftz*, (1.9 kb); *zen* (1.3 kb); *zen2* (1.0 kb) and *bcd* (3.6 kb) [5]. These compact homeobox TFs regulate the pair-rule segmentation cascade (*ftz*), mitotic domain δ14-1, the optic lobe of the CNS, the dorsal ridge and axial cell fates (*zen, zen2*), and the A > P morphogenetic gradient (*bcd*). Other compact transcripts encode a cluster of 7 cuticle proteins, *ccp84Aa-g* (0.64 to 1.3 kb) and the neurotactin ligand, *ama* (3.7 kb). By contrast, the *Bx-C* contains only three Hox TFs (*Ubx*, *abd-A* and *Abd-B*), which between them regulate the fate of the three thoracic and nine abdominal segments. An additional compact transcript nested within *bxd* encodes the Dynein light chain (*CG31275*, 0.59 kb), with the initial protease in the Toll-mediated immune response, *ModSP* (4.5 kb) immediately proximal to *Ubx*. These additional genetic functions may be co-regulated with adjacent Hox transcripts, if only to the extent of being affected by chromatin compaction and the Pc/Trx balance.

The Hox complexes also include multiple microRNAs and long non-coding RNAs (*miR*s and *lncRNAs*). In general, *miR* transcripts form 60-120 bp hairpin loops, which are processed to the 20-22 bp fragments that regulate complex transcriptional networks [31 32 33]. As might be expected, some *Hox-C miR*s regulate their adjacent TUs, as well as more distant genetic functions [34 35 36]. In the *Ant-C*, the *miR-10* and *miR-993* transcripts (76 bp and 119 bp) are nested within *lncRNA*s (10.6 kb and 17.8 kb) [37 38], which may impose additional regulatory constraints. Within the *Bx-C*, the *bithoraxoid* (*bxd*) *lncRNA* (43.5 kb), regulates adjacent *Ubx* and *abd-A* transcripts [39 40]. The Dynein light-chain (*CG31275*) is conserved within the Diptera, which might be consistent with a function related to the reduced growth of the metathoracic wing (haltere). Meanwhile, the differential fates of the abdominal segments are regulated by the *infra-abdominal-4* (*iab-4*) and *iab-8* TUs, which separate the *abd-A* and *Abd-B* genes [41 42 34]. Mir-8 *Iab-4* (9.1kb) is expressed at high levels in A5-A7, and represses *abd-A* expression; while *iab-8* (129 kb) is expressed in A8 and A9 [43]. The *iab-4* transcript itself is nested within an intron of *iab-8* but is transcribed in the opposite orientation. However, both transcripts are spliced to produce the same 68 bp hairpin loop (*miR-iab-4*), which is further processed into two distinct 22 bp fragments [33]. These 22bp regulatory fragments have differential binding affinities to target sites within the 3' UTRs of *Antp*, *Ubx* and *abdA* [33 44]. Thus, despite being processed to the same active 22 bp fragments, the *iab-4* and *iab-8* functions generate differential responses from their adjacent transcriptional targets. The basis for these altered responses is uncertain, but consistent with differential temporal transcription patterns of the sense and antisense DNA strands. Outside the *Bx-C*, the 22 bp *miR-iab-4* fragments target the 3' UTRs of the *hth* and *extradenticle* Hox co-factors [45 46]. By contrast, the *miR-10* transcript within the *Antp-C* affects wing venation. Notably, mammalian *miR-10* orthologues are present in each of the four paralogous Hox gene complexes, where they regulate adjacent Hox functions, in addition to Wnt signalling and cancer metastasis [38 47 37]. Nested *miR* transcripts within *lncRNAs* are also found outside the *Antp*- and Bx- complexes in *Drosophila*. For example, *miR-184* TU (61 bp) maps within *lncRNA-CR44206* (21.6 kb), with regulatory functions in germline stem-cell differentiation (via the Dpp receptor, Saxophone); D/V patterning (via the Grk transport factor K10); A/P patterning (via Tramtrack69) and the Cad gradient [48]. Similarly, the *miR-7* and *miR-8* nested transcripts activate tumorigenesis (via *N*, *wg* and *hh*) and *Tl, dll, wg* and *ena*, respectively [44 49 50 51]. In addition, the *miR-310-313* gene cluster modulates Wg signalling through the 3′UTRs of *arm* and *pangolin* [52] and *miR-310C* regulates *Ubx* expression and adult behavioural responses [53]. Thus, the networks of transcriptional regulation are bewilderingly complex, with component TUs scattered throughout the genome. However, as first described by E.B. Lewis, the co-linear organisation of the Hox regulatory hubs remains elegantly simple.

Summary:

The Hox gene complexes implement the maternal pre-patterning of the oocyte as the zygotic genome is fully activated. The chromosomal organisation of Hox gene functions is co-linear with their regional expression domains, with interspersed *miRs*, *lncRNAs* and additional, compact protein-coding genes. Notably, the single Hox gene cluster of *Drosophila* is separated into and A and P clusters (Ant-C and Bx-C) with overlapping domains across the A/P equatorial midline. Additional, compact homeobox TFs in the Ant-C regulate pair-rule expression, the A > P morphogenetic gradient and D/V fate. Thus, the Hox gene clusters correspond to genetic regulatory hubs that integrate global patterns of chromatin compaction, with transcriptional regulation of morphogenetic functions scattered throughout the genome

References:

1. Lewis, E. B. A gene complex controlling segmentation in Drosophila. *Nature* **276**, 565 (1978).
2. Levine, M., Hafen, E., Garber, R. L. & Gehring, W. J. Spatial distribution of Antennapedia transcripts during Drosophila development. *EMBO J.* **2**, 2037–2046 (1983).
3. Lewis, E. B. Regulation of the genes of the Bithorax complex in Drosophila. *Cold Spring Harb. Symp. Quant. Biol.* **50**, 155–164 (1985).
4. Martinez Arias, A. & Lawrence, P. A. Parasegments and compartments in the Drosophila embryo. *Nature* **313**, 639–642 (1985).

5. 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).
6. Lewis, E. B. The relation of repeats to position effect in Drosophila melanogaster. *Genetics* **30**, 137 (1945).
7. Gemkow, M. J., Verveer, P. J. & Arndt-Jovin, D. J. Homologous association of the Bithorax-Complex during embryogenesis: consequences for transvection in Drosophila melanogaster. *Development* **125**, 4541 (1998).
8. Kennison, J. A. & Southworth, J. W. Transvection in Drosophila. *Adv. Genet.* **46**, 399–420 (2002).
9. Chalkley, G. E. *et al.* The transcriptional coactivator SAYP is a trithorax group signature subunit of the PBAP chromatin remodeling complex. *Mol. Cell. Biol.* **28**, 2920–2929 (2008).
10. Kassis, J. A. Transvection in 2012: site-specific transgenes reveal a plethora of trans-regulatory effects. *Genetics* **191**, 1037 (2012).
11. Nègre, N. *et al.* A cis-regulatory map of the Drosophila genome. *Nature* **471**, 527 (2011).
12. Kharchenko, P. V. *et al.* Comprehensive analysis of the chromatin landscape in Drosophila. *Nature* **471**, 480–485 (2011).
13. Gramates et al. FlyBase: a guided tour of highlighted features. *Genetics* (2022).
14. Khoroshko, V. A. *et al.* Genes containing long introns occupy series of bands and interbands in Drosophila melanogaster polytene chromosomes. *Genes* **11**, (2020).
15. Beuchle, D., Struhl, G. & Muller, J. Polycomb group proteins and heritable silencing of Drosophila Hox genes. *Development* **128**, 993 (2001).
16. Bowman, S. K. *et al.* H3K27 modifications define segmental regulatory domains in the Drosophila bithorax complex. *eLife* **3**, e02833 (2014).
17. Klymenko, T. *et al.* A Polycomb group protein complex with sequence-specific DNA-binding and selective methyl-lysine-binding activities. *Genes Dev.* **20**, 1110–1122 (2006).
18. Czermin, B. *et al.* Drosophila Enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. *Cell* **111**, 185–196 (2002).
19. Tie, F. *et al.* Trithorax monomethylates histone H3K4 and interacts directly with CBP to promote H3K27 acetylation and antagonize Polycomb silencing. *Development* **141**, 1129 (2014).
20. Kassis, J.A. Pairing-sensitive silencing, Polycomb group response elements, and transposon homing in Drosophila. *Adv Genet.* **46**, 421–438 (2002).
21. Martinez, A.-M., Colomb, S., Déjardin, J., Bantignies, F. & Cavalli, G. Polycomb group-dependent Cyclin-A repression in Drosophila. *Genes Dev.* **20**, 501–513 (2006).
22. Nègre, N. *et al.* A cis-regulatory map of the Drosophila genome. *Nature* **471**, 527–531 (2011).
23. Rinn, J. L. *et al.* Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. *Cell* **129**, 1311–1323 (2007).
24. Soshnikova, N. & Duboule, D. Epigenetic temporal control of mouse Hox genes in vivo. *Science* **324**, 1320 (2009).
25. Morata, G. & Kerridge, S. Sequential functions of the bithorax complex of Drosophila. *Nature* **290**, 778–781 (1981).
26. Casares, F. & Sanchez-Herrero, E. Regulation of the infraabdominal regions of the bithorax complex of Drosophila by gap genes. *Development* **121**, 1855 (1995).

27. Pignoni, F. *et al.* The Drosophila gene tailless is expressed at the embryonic termini and is a member of the steroid receptor superfamily. *Cell* **62**, 151–163 (1990).
28. Shokri, L. *et al.* A comprehensive Drosophila melanogaster transcription factor interactome. *Cell Rep.* **27**, 955-970.e7 (2019).
29. Wu, X., Vasisht, V., Kosman, D., Reinitz, J. & Small, S. Thoracic patterning by the Drosophila gap gene hunchback. *Dev. Biol.* **237**, 79–92 (2001).
30. Estrada, B. & Sanchez-Herrero, E. The Hox gene Abdominal-B antagonizes appendage development in the genital disc of Drosophila. *Development* **128**, 331 (2001).
31. Lee, R. C. & Ambros, V. An Extensive Class of Small RNAs in Caenorhabditis elegans. *Science* **294**, 862 (2001).
32. Brennecke, J, Stark, A, & Russel, RB. Principles of MicroRNA–Target Recognition. *PLoS Biol 33 E85* **3(3)**, (2005).
33. Stark, A. *et al.* A single Hox locus in Drosophila produces functional microRNAs from opposite DNA strands. *Genes Dev.* **22**, 8–13 (2008).
34. Bender, W. MicroRNAs in the Drosophila bithorax complex. *Genes Dev.* **22**, 14–19 (2008).
35. Yekta, S., Tabin, C. J. & Bartel, D. P. MicroRNAs in the Hox network: an apparent link to posterior prevalence. *Nat. Rev. Genet.* **9**, 789–796 (2008).
36. Garaulet, D. L. *et al.* Homeotic function of Drosophila Bithorax-complex miRNAs mediates fertility by restricting multiple Hox genes and TALE cofactors in the CNS. *Dev. Cell* **29**, 635–648 (2014).
37. Tehler, D., Høyland-Kroghsbo, N. M. & Lund, A. H. The miR-10 microRNA precursor family. *RNA Biol.* **8**, 728–734 (2011).
38. Lund, A. H. miR-10 in development and cancer. *Cell Death Differ.* **17**, 209 (2009).
39. Bae, E., Calhoun, V. C., Levine, M., Lewis, E. B. & Drewell, R. A. Characterization of the intergenic RNA profile at abdominal-A and Abdominal-B in the Drosophila bithorax complex. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 16847–16852 (2002).
40. Petruk, S. *et al.* Transcription of bxd noncoding RNAs promoted by trithorax represses Ubx in cis by transcriptional interference. *Cell* **127**, 1209–1221 (2006).
41. Celniker, S. E. & Lewis, E. B. Molecular basis of transabdominal--a sexually dimorphic mutant of the bithorax complex of Drosophila. *Proc. Natl. Acad. Sci. U. S. A.* **90**, 1566–1570 (1993).
42. Karch, F, Bender, W, & Weiffenbach, B. abdA expression in Drosophila embryos. *Genes Dev.* **4**, 1573–1587 (1990).
43. Gummalla, M. *et al.* abd-A Regulation by the iab-8 Noncoding RNA. *PLoS Genet.* **8**, e1002720 (2012).
44. Biemar, F. *et al.* Spatial regulation of microRNA gene expression in the Drosophila embryo. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 15907–15911 (2005).
45. Garaulet, D. L. *et al.* Homeotic function of Drosophila Bithorax-complex miRNAs mediates fertility by restricting multiple Hox genes and TALE cofactors in the CNS. *Dev. Cell* **29**, 635–648 (2014).
46. Garaulet, D. L. & Lai, E. C. Hox miRNA regulation within the Drosophila Bithorax Complex: patterning behavior. *Mech. Dev.* **138**, 151–159 (2015).
47. Stadthagen, G. *et al.* Loss of miR-10a Activates Lpo and Collaborates with Activated Wnt Signaling in Inducing Intestinal Neoplasia in Female Mice. *PLoS Genet.* **9**, e1003913 (2013).
48. Iovino, N., Pane, A. & Gaul, U. miR-184 has multiple roles in Drosophila female germline development. *Dev. Cell* **17**, 123–133 (2009).

49. Da Ros, Gutierrex-Perez, Irene, Ferres-Marco, Dolors, & Dominguez, Maria. Dampening the signals transduced through Hedgehog via MicroRNA miR-7 facilitates Notch-Induced tumourigenesis. *PLoS Biol.* **11**, (2013).
50. Vallejo, D. M., Caparros, E. & Dominguez, M. Targeting Notch signalling by the conserved miR-8/200 microRNA family in development and cancer cells. *EMBO J.* **30**, 756–769 (2011).
51. Nesler, K. R. *et al.* The miRNA pathway controls rapid changes in activity-dependent synaptic structure at the Drosophila melanogaster neuromuscular junction. *PLoS ONE* **8**, e68385 (2013).
52. Pancratov, R. *et al.* The miR-310/13 cluster antagonizes β-catenin function in the regulation of germ and somatic cell differentiation in the Drosophila testis. *Development* **140**, 2904 (2013).
53. Kaschula, R., Pinho, S. & Alonso, C. R. MicroRNA-dependent regulation of Hox gene expression sculpts fine-grain morphological patterns in a Drosophila appendage. *Development* **145**, dev161133 (2018).