

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.

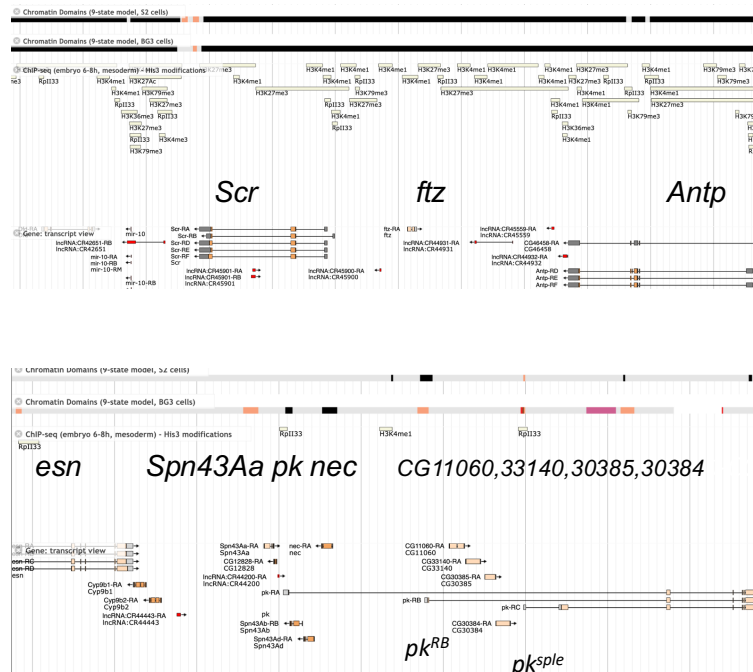


Fig. 31. Hox complexes genes are late replicating, hypermethylated and PC repressed. A. The *scr*, *ftz* and *Antp* TUs. However, extended genes outside the Hox complexes may have Pc-repressed exons, but their intronic segments, and any nested transcripts, tend not to be. For example: **B.** The coding 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 fates²⁵. 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 ridge²⁶. 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 PolIII-specific hormone response element (HRE), initially at both embryonic poles, but later restricted to the P pole and the AD midline²⁷. 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 receptor²⁸. 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 segments³⁰.

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 δ 14-1, 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 (*miRs* 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* *miRs* 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 *lncRNAs* (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 (halter). 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}. *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 A9⁴³. 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 fragments³³. 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 complementary 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 gradient⁴⁸. 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 responses⁵³. 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 an 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

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