Chapter 19. Developmental switches, splicing factors and single-base DNA alterations.

The gap, segmentation and homeotic genes delineate AMSs and segmental boundaries in *Drosophila*. In addition, to allocating parasegmental fate, these genetic functions regulate the progression of segmental identity (and termination of the segmental chain); with an analogous set of interactions during limb growth. However, the best-studied morphogenetic switching mechanism may be the sex determination cascade. The program required to construct male legs differs only slightly from that deployed to make female legs: muscles are built, tendons connected, and multiple metabolic functions co-ordinately regulated. Fine-grained, dynamic changes in the activities of large sets of genes must be integrated; while alternative, gender-specific, patterns of expression are suppressed. Slightly different configurations of downstream genetic functions must be deployed in the pro-, meso- and meta-thoracic leg discs of male and female *Drosophila*, without interfering with their assigned gender. Similarly, the individual segments of jointed limbs must deploy similar sub-sets of downstream gene activities, in all six legs and in both sexes. Many of the same functions will be required in the dorsal notum and wing blade. How such complex regulatory networks might be assembled over evolutionary timescales remains enigmatic.

The global regulation of gender-specific fate is controlled via the *sex-lethal*, *transformer* and *transformer-2* splicing factors [1] [2] [3]. In particular, sexually dimorphic terminal abdominal fate is regulated via alternatively spliced transcripts of the *Bx-C*, *abd-A* and *Abd-B*, which are differentially expressed in males and females [4] [5]. By comparison, the *Suppressor of white-apricot* splicing factor, *Su(w^a)*, modifies the splicing rate of particular introns in several target genes [6], and auto-regulates maturation of its own transcripts [7] [8]. Taken together, these observations suggest a more general role for sequence-specific splicing factors. Notably, the Su(w^a) protein binds to Rictor (Rapamycin insensitive companion of Tor), controlling the phosphorylation of kinases regulating growth and the insulin signalling pathway [9]. The orthologous mammalian Su(w^a*)* protein regulates alternative splicing of exon 4 of *CD45* and tissue-specific splicing of *fibronectin* [10], and presumably other target transcripts that have yet to be identified. Sequence-specific factors that regulate splicing-delay might alter the balance between the alternative protein isoforms, or functions encoded by separate genetic loci. Any gender-specific splicing patterns must also be responsive to global metabolic controls. By implication, TFs, growth factors (GFs), morphogens and chromatin remodelling functions may all be targets of sequence-specific splicing factors. In addition, downstream metabolic functions could be individually modulated by splicing factors, without disrupting the regulation of core morphogenetic functions. In this context, single-base changes near splice-donor or -acceptor sites may modify splicing factor binding affinity. Such single-base changes would allow individual genetic functions to be added to, or removed from, the set of transcripts regulated by a particular splicing factor, without altering the peptide sequence of the translated protein. More generally, single-base alterations at TF binding sites may modify promotor binding affinity, as well as the transcription rate of intronic segments, transcript maturation at 3' UTRs and intragenic regulatory domains. In particular, single base changes at *microRNA* (*miR*) target sites may alter transcript maturation rate and perdurance. Similarly, single base changes at putative SLiM target sites may alter the post-translational modification of protein activities.

Thus, single-base alterations may be stably inherited from one generation to the next, while remaining labile over evolutionary timescales. This ontogenetic stability would allow the rapid, chaotic assembly of multi-layered regulatory networks, with only rare amino-acid substitutions within protein-coding motifs. An emergent property of such increasing complexity is that morphogenetic pathways would become buffered. Core regulatory

interactions may tend to be conserved, while single-base alterations may be complemented by balancing alterations within other genetic functions. The potential set of integrated, morphogenetic pathways is unlimited, although assigning a precise function to individual morphogenetic TUs is rarely possible.

Summary:

Sequence-specific splicing factors may coordinate switching between alternative, metastable pathways, particularly during sex-determination. The balance between different protein activities may be altered by single-base DNA alterations, which would rarely alter the encoded peptide sequence. The cumulative effect of single-base changes allows complex morphogenetic programs to be assembled over evolutionary timescales. In consequence, core morphogenetic interactions may be strongly conserved between multicellular organisms, while any limits on the complexity of developmental pathways are indeterminate.

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