Chapter 14. Morphogenetic "blind-spots" and the limitations of genetic analysis.

Genetic analysis has been a powerful tool for elucidating metabolic pathways; with amorphic, lack of function (LOF), mutations defining sequential enzymatic steps [1] [2] [3]. While homozygous LOF mutations may block an enzymatic pathway, the partial reduction of enzyme activity in heterozygous mutants may lead to substrate accumulation. Thus, the resulting shift in binding kinetics may increase the activity of the remaining enzyme pool, with little reduction in metabolic flux. In consequence, null mutations in enzymatic pathways are generally recessive. Similarly, increased expression of a metabolic enzyme rarely causes a visible phenotype. By contrast, null morphogenetic mutations may be complemented by related genetic functions, or drive mis-regulated responses. Unlike metabolic enzymes, the overexpression of morphogenetic functions tends to cause dominant gain of function (GOF) phenotypes, as signalling networks adjust to alternative metastable configurations. Indeed, most of the classical dominant mutants of *Drosophila* are associated with GOF mutations of morphogenetic functions. Notably, over-expression of segmentation gene functions gives phenotypes similar to their corresponding LOF mutations, but with pattern reversals that are 90º out of phase [4] [5]. Similarly, overexpression of PCP genes gives domineering polarity alterations that are the reverse of those associated with LOF mutations [6] [7] [8].

In classical genetic terminology, morphogenetic mutations tend to be "redundant" and "pleiotropic", although these concepts remain poorly defined at the molecular level. Furthermore, interrelated morphogenetic activities may become "entangled" to the extent that the primary function of individual genes is uncertain; a striking example being *Cdc42* (see above, **4**). The resultant phenotypic alterations can be difficult to interpret, particularly in the case of cognate-twin transcripts, such as *engrailed* (*en*) and *invected* (*inv*). In this example, the *en* gene regulates posterior compartmental identity and A/P boundary formation [9]. The mutant phenotype of homozygous en^1 flies includes a partial P > A transformation of the adult wing. This transformation is reduced when en^1 is heterozygous with a chromosomal deletion ($en^1/Df(2R)en^-$), and the adult phenotype is close to wild-type when en^1 is heterozygous with a lethal null allele ($en^1/l(2)en^-$) [10]. However, both en^1 and $l(2)en^-$ clones may cross the A/P compartment boundary, but only from P > A [9] [11]. Both *en* and *inv* activities are reduced by the en^1 mutation, but a complete P > A transformation is produced with transplanted *inv en* double-mutant discs cultured in wild-type host larvae [12] [13]. By implication, the incomplete P > A transformation in en^1 wings is largely complemented by the adjacent *inv* gene activity. By contrast, the en^{28} mutant transforms wing tissue to haltere in the middle of the wing blade [14]. Heterozygous $Tp(2;3)en^{28}/Df(2R)inv^- en^-, en^{30}$ wings show no P > A transformation, although wing venation is disrupted in both A and P compartments, and haltere tissue appears to straddle the A/P mid-line (Fig. 19).

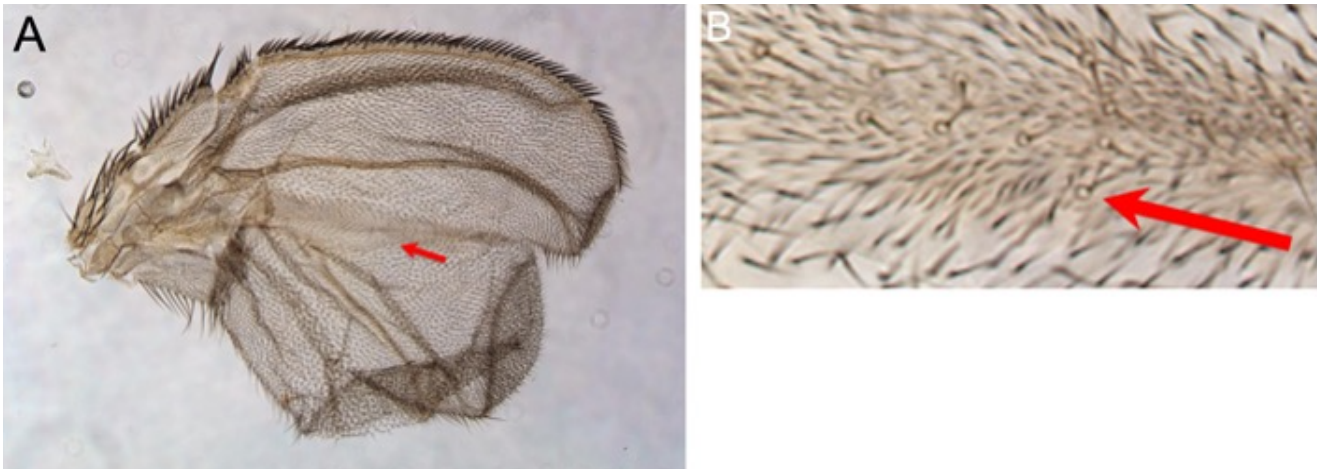

Fig. 19. Haltere tissue in en^{28} wings. A. A strip of compact cells straddles the A/P midline, with vein malformations in both wing compartments and no ectopic bristles on P wing margin, $Tp(2;3)en^{28}/Df(2R)inv^- en^-, en^{30}$ mutant. **B.** Enlargement of region spanning the A/P boundary of en^{28} wing. Red arrow indicates haltere-like sensillae. D. Gubb, unpublished, see also [14].

Furthermore, large clones of $Df(2R)enE, inv^- en^-$ can cause mirror-image duplications of the entire wing blade, multiple A veins in the P wing, or P veins in the A wing [15]. These mutant defects presumably

reflect altered transcription of the *inv* and *en* genes, which may be juxtaposed to ectopic regulatory segments at the *Tp(2;3)en*^{28} aberration breakpoints. Complex interrelated functions are also shown by the Toll (Tl) signalling pathway that regulates the D/V (L/R) embryonic axis, in addition to the innate immune response, cardio-vascular development, haematopoiesis and synapse function [16 17 18 19 20]. The *Tl* gene does not have an adjacent cognate function, but nine Toll-like (Tll) receptors exist at other chromosomal loci, including three adjacent transcripts (*Tll-2*, *Tll-6* and *Tll-8*). Removal of more than one of these *Tll* transcripts gives defects in segmentation, convergent extension and remodelling of the actin cytoskeleton [21]. Clearly, the pleiotropic functions of *Tl* and the *Tll* receptors entrain multiple morphogenetic pathways, with partial complementation between LOF mutations.

Summary:

The sequential steps in metabolic pathways may be defined by LOF mutations. However, LOF mutations in morphogenetic pathways may be complemented by related genetic functions or disrupt alternative signalling pathways. Thus, dominant GOF phenotypes tend to be associated with morphogenetic functions. Furthermore, entangled genetic interactions may prevent the identification of some functions, while mutant alleles may switch between alternative developmental pathways. In this context, adjacent TUs may share overlapping regulatory domains, although most regulatory interactions take place between widely separated loci. Genetic analysis may fail to identify all morphogenetic functions, while the precise role of individual TUs remains masked and indeterminate.

References:

1. Garrod, A. *Inborn Errors of Metabolism*. (Hodder & Stoughton, London, 1923).
2. Muller, H. J. Further studies on the nature and causes of gene mutations. *Proc. Sixth Int. Congr. Genet. Ithaca N. Y.* **1**, 213–255 (1932).
3. Sullivan & Sullivan. Transport defects as the physiological basis for eye color mutants of Drosophila melanogaster. *Biochem Genet* **13**, 603–613 (1975).
4. Gergen, J. P. & Wieschaus, E. F. The localized requirements for a gene affecting segmentation in Drosophila: analysis of larvae mosaic for runt. *Dev. Biol.* **109**, 321–335 (1985).
5. Struhl, G. Near-reciprocal phenotypes caused by inactivation or indiscriminate expression of the Drosophila segmentation gene ftz. *Nature* **318**, 677–680 (1985).
6. Krasnow, R. E. & Adler, P. N. A single frizzled protein has a dual function in tissue polarity. *Development* **120**, 1883–1893 (1994).
7. Axelrod, J. D., Miller, J. R., Shulman, J. M., Moon, R. T. & Perrimon, N. Differential recruitment of Dishevelled provides signaling specificity in the planar cell polarity and Wingless signaling pathways. *Genes Dev.* **12**, 2610–2622 (1998).
8. Gubb, D. *et al.* The balance between isoforms of the prickle LIM domain protein is critical for planar polarity in Drosophila imaginal discs. *Genes Dev.* **13**, 2315–2327 (1999).
9. Lawrence, P. A. & Morata, G. Compartments in the wing of Drosophila: a study of the engrailed gene. *Dev. Biol.* **50**, 321–337 (1976).
10. Gubb, D. Further studies on engrailed mutants in Drosophila melanogaster. *Rouxs Arch. Dev. Biol.* **194**, 236–246 (1985).
11. Kornberg, T. Engrailed: a gene controlling compartment and segment formation in Drosophila. *Proc. Natl. Acad. Sci. U. S. A.* **78**, 1095–1099 (1981).
12. Simcox, A. A. *et al.* Imaginal discs can be recovered from cultured embryos mutant for the segment-polarity genes engrailed naked and patched but not from wingless. *Development* **107**, 715–722 (1989).
13. Gustavson, E., Goldsborough, A. S., Ali, Z. & Kornberg, T. B. The Drosophila engrailed and invected genes: Partners in regulation, expression and function. *Genetics* **142**, 893–906 (1996).
14. Eberlein, S. & Russell, M. A. Effects of deficiencies in the engrailed region of Drosophila melanogaster. *Dev. Biol.* **100**, 227–237 (1983).
15. Tabata, T., Schwartz, C., Gustavson, E., Ali, Z. & Kornberg, T. B. Creating a Drosophila wing de novo, the role of engrailed, and the compartment border hypothesis. *Development* **121**, 3359 (1995).

16. Anderson, K. V. & Nusslein-Volhard, C. Genetic analysis of the dorso-ventral embryonic pattern in Drosophila. *Pattern Form. Primer Dev. Biol.* 269–289 (1984).
17. Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J. M. & Hoffmann, J. A. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. *Cell* **86**, 973–983 (1996).
18. Meister, M., Hetru, C. & Hoffmann, J. A. The antimicrobial host defense of Drosophila. *Orig. Evol. Vertebr. Immune Syst.* **248**, 17–36 (2000).
19. Suzuki, E., Rose, D. & Chiba, A. The ultrastructural interactions of identified pre- and postsynaptic cells during synaptic target recognition in Drosophila embryos. *J. Neurobiol.* **42**, 448–459 (2000).
20. Wang, J. *et al.* Expression, regulation, and requirement of the toll transmembrane protein during dorsal vessel formation in Drosophila melanogaster. *Mol. Cell. Biol.* **25**, 4200–4210 (2005).
21. Paré, A. C. *et al.* A positional Toll receptor code directs convergent extension in Drosophila. *Nature* **515**, 523–527 (2014).