Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

Discovery and Genetic Definition of the Drosophila Antennapedia Complex

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ONE day in 1976 THOM KAUFMAN called me in great excitement. KAUFMAN and I shared an interest in Drosophila homeotic genes located near the third chromosome centromere. He had recently arrived at Indiana University, and the results of his first set of crosses convinced him that these genes were part of a complex important to anterior developmental commitments (KAUFMAN *et al.* 1980), just as ED LEWIS's bithorax complex controlled fate in more posterior regions. He invited me to Bloomington the next summer to join him in a genetic study of this Antennapedia complex (ANT-C).

In the late 1960s, KAUFMAN and I had been graduate students together in BURKE JUDD's lab at the University of Texas. Although my interest in segregation distortion was far from the focus of the lab, KAUFMAN worked at its very center: a mutational analysis of the *zeste-white* region. JUDD was and remains interested in genome organization, and in this pre-molecular era he wished to ascertain all zygotically active functions in this *X* chromosome interval necessary for normal morphology and/or viability and to examine the distribution of these loci along the polytene chromosome map (JUDD *et al.* 1972). In addition to the genetic aspects of this "saturation mutagenesis" effort, KAUFMAN joined MARY SHANNON in a study of the developmental consequences of the recessive-lethal variants isolated (SHANNON *et al.* 1972).

I left Austin in 1970 for postdoctoral studies with DAN LINDSLEY at UCSD and arrived there at a particularly exciting time. LINDSLEY and long-time collaborator LARRY SANDLER were also interested in functional aspects of genetic organization, and I joined their massive effort to systematically generate and study small deficiencies and duplications distributed throughout the genome (LINDSLEY *et al.* 1972). LINDSLEY has served as a repository and chronicler of Drosophila genetic knowledge and takes seriously his responsibility to impart this lore to his students. He pointed out to me an apparent enrichment of homeotic and sex-transforming genes near the third chromosome centromere. These genes were fascinating from a developmental standpoint, and it had been argued for decades that they must play key regulatory roles in assigning developmental commitments realized through the action of downstream genes. Moreover, the apparent clustering of genes of similar function in a higher eukaryote was potentially very interesting. However, uncertainties as to allelic relationships and mapping difficulties due to centromeric inhibition of recombination in this region (HANNAH-ALAVA 1969) left the number of homeotic genes and their spatial relationships in considerable doubt.

All known homeotic loci in that region had been recognized by adult transformations, and included *Extra sex combs* (*Scx*), *proboscipedia* (*pb*), *Antennapedia* (*Antp*), *Polycomb* (*Pc*), *Multiple sex combs* (*Msc*), and *Nasobemia* (*Ns*) (Figure 1A). [That *Deformed* (*Dfd*) and *Humeral* (*Hu*) were homeotic mutations was not then recognized.] Many *Antennapedia* mutant alleles shared a common dominant antenna → leg transformation phenotype, rearrangement breakpoint, and recessive lethality. WALTER GEHRING (1966) had discovered a mutation associated with a similar (albeit more extreme) transformation that was free of recessive lethality and gross chromosomal rearrangement. He believed this variant to identify a locus separate from *Antennapedia* and named it *Nasobemia* after MORGENSTERN's mythological creature that walked on its nose. ELIEZER LIFSCHYTZ, then also in LINDSLEY's lab, had recently shown that the dominant phenotype of a gain-of-function allele could be "reverted" by loss-of-function mutations isolated at rates characteristic of forward mutation (LIFSCHYTZ and FALK 1969). LINDSLEY suggested that I use LIFSCHYTZ's approach for the sex-transforming mutation then known as *transformer-dominant* (which proved to be *doublesex-dominant* instead), and I also applied it to *Nasobemia*. Most revertants were associated with recessive lethality that failed to complement the lethality of *Antennapedia* mutations, showing that *Nasobemia* was an *Antennapedia* allele and that the phenotype that was its namesake was neomorphic (DENELL 1973). *Extra sex combs* appeared to be an *Antennapedia* allele as well. A

very similar reversion analysis was done independently by KAUFMAN and IAN DUNCAN, then an undergraduate at the University of British Columbia (DUNCAN and KAUFMAN 1975). [In this issue, TALBERT and GARBER (1994) discuss the molecular nature of *Nasobemia* revertants.] These results clarified the picture a little, but the question of relationships in the genome and their potential significance remained unsolved.

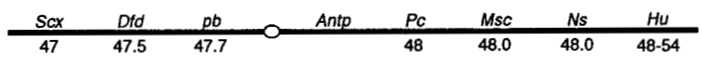
At this time ED LEWIS was performing his well known and elegant work at Caltech on other homeotic mutations. In this case, a large number of recessive and dominant variants causing homeotic transformations of the adult thorax and abdomen were unambiguously very tightly clustered within the bithorax complex (BX-C), which mapped more distally on the right arm of chromosome *3*. LEWIS (1978) argued that loss-of-function variants caused anteriorly directed transformations, whereas those associated with gain-of-function variants were posteriorly directed. Moreover, the mutations mapped along the chromosome in an order colinear with their mutant effects along the anterior-posterior body axis. This distribution led Lewis to the hypothesis that the number of genes corresponded to the number of thoracic and abdominal segments. He suggested a model in which the middle thoracic segment was a "ground state" characteristic of no BX-C activity in the context of an otherwise normal genotype, and the expression of a progressively greater number of BX-C genes defined successively more posterior segments. He interpreted gain-of-function mutations as being due to the expression of a gene in an inappropriate domain. For me at least, this idea provided one satisfying explanation for the difficult question of how neomorphic mutations could acquire "a new function" (MULLER 1932). Noting that Japanese geneticists studying the silk moth equivalent of the BX-C had placed emphasis on larval mutant phenotypes (TAZIMA 1964), LEWIS also stressed the importance of examining the terminal lethal syndromes of genotypes dying at preadult stages.

KAUFMAN's first crosses at Indiana used a small deficiency isolated as a revertant of *Nasobemia* (*Df(3R)Ns+R17*), and an overlapping deletion isolated at the University of British Columbia by DON SINCLAIR called *Df(3R)Scr*. The latter was associated with a reduction in the size of the sex combs of the male proleg shared by *Multiple sex combs*, and KAUFMAN postulated that deletion of a haplo-insufficient *Sex combs reduced* gene caused a partial transformation of first to second leg. Complementation analysis using the deficiencies indicated that polytene chromosome region 84A-B included all of the homeotic loci known in the proximal third chromosome region except *Polycomb*, which by then had been shown to reside on the other side of the centromere (PURO and NYGREN 1975). This exciting definitive evidence of clustering led Kaufman to propose that these genes were part of a complex controlling anterior determinative decisions, and to predict similarities to the BX-C. He envisaged the middle thoracic segment as a ground state, such that the loss-of-function phenotype of *Sex combs reduced* caused posteriorly directed changes of the first thoracic leg, and dominant gain-of-function variants *Multiple sex combs* and *Extra sex combs* caused opposing changes (KAUFMAN *et al.* 1980).

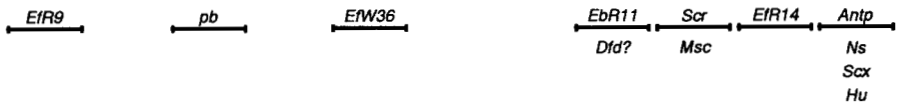
How could the structure and function of this complex be elucidated? KAUFMAN recognized that the available collection of variants, isolated on the basis of adult homeotic phenotypes, probably had been strongly influenced by ascertainment bias. Except for some *proboscipedia* alleles, all available visible mutations were neomorphs, and null alleles were needed to assess their normal developmental significance. Moreover, he anticipated that additional functionally related genes existed within the complex that could be recognized by the embryonic or larval phenotypes of recessive lethal alleles. Thus, he proposed that we apply JUDD's concept of saturation mutagenesis to identify and characterize all loci in this region that mutate to yield recessive lethal or visible adult phenotypes. When I arrived in Bloomington in the summer of 1976, KAUFMAN and graduate student RICKI LEWIS had set up an experiment to isolate X-ray- and EMS-induced mutations that failed to complement $Df(3R)Ns^{+R17}$. Later, graduate student BARBARA WAKIMOTO screened for additional mutations using *Df(3R)Scr*. Both sets of variants were subjected to complementation and recombinational analysis, and the map shown in Figure 1B was generated (LEWIS *et al.*, 1980a,b). As predicted, point mutations associated with a dominant adult T1 → T2 transformation were isolated at the *Sex combs reduced* locus. The results also suggested that the *EbR11* complementation group included the *Deformed* mutation, and preliminary observations showed that the lethal syndromes associated with the *EbR11* and *EfW36* complementation groups included larval head abnormalities.

The description of complementation groups with arbitrary names generated little widespread excitement. The real payoff began when WAKIMOTO and KAUFMAN (1981) and WAKIMOTO *et al.* (1984) described the phenotypes of lethal larvae and adult clones homozygous for recessive lethal alleles (see KAUFMAN *et al.* 1990). KAUFMAN (1978) had earlier made the unexpected observation that *proboscipedia* function is dispensable for normal embryonic development, so it was satisfying that the null phenotypes of *Antennapedia* and *Sex combs reduced* were associated with larval homeotic transformations. The *EfR14* complementation group was associated with aberrant embryos missing half of the normal number of segments. Wishing to give this gene a descriptive Japanese name, WAKIMOTO and KAUFMAN consulted colleague BOB TOGASAKI as well as WAKIMOTO's father and settled on *fushi tarazu* (*ftz*), a term that roughly translates to describe a shortening of bamboo by loss of segments. This segmentation gene has been among the most intensively studied in Drosophila. They also found that the *EfW36* complementation group caused abnormalities in

A. Recombinational map positions, from LINDSLEY and GRELL (1967)



B. Complementation groups, after LEWIS *et al.* (1980a)



C. Post-molecular organization, after KAUFMAN *et al.* (1990)

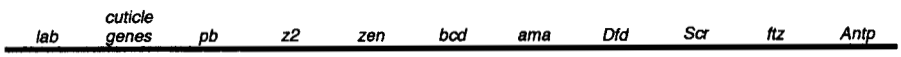


FIGURE 1.—Conceptual evolution of the Drosophila Antennapedia complex. (A) Map positions of the homeotic mutations (as well as *Deformed* and *Humeral*) recognized in 1967 in the proximal region of chromosome *3*, with the centromere position indicated by a circle. Because of centromeric inhibition of recombination, the region from *Scx* to *Ns* potentially spanned salivary chromosome map region 77–84. All recognized *Antp* alleles were associated with rearrangements sharing breakpoints in proximal 84, and its relative position was assigned on that basis. Likewise, *Humeral* is associated with an inversion of salivary chromosome map region 84–86 and was placed at recombinational map position 48–54. Later studies would show that *Pc* is in the left arm and all others in the right arm at salivary map position 84B1,2. (B) Complementation groups recognized by LEWIS *et al.* (1980a). They have been aligned with the current version of the map (based on molecular as well as genetic studies) shown in (C), although LEWIS *et al.* showed them in a slightly different order.

morphogenetic rearrangements associated with gastrulation, and renamed the locus *zerknüllt* (*zen*) (German for crumpled). Later work has defined the importance of this gene in dorsal-ventral patterning. Studies of the *EbR11* complementation group (later to be *Deformed*) detected anterior abnormalities that were not obviously homeotic in nature, and later work on the *EfR9* or *labial* (*lab*) group also demonstrated similar nonhomeotic head defects.

Which of these loci associated with diverse mutant phenotypes potentially belonged to a complex of functionally related genes? KAUFMAN argued that all of the genes necessary for early embryonic development (as well as *proboscipedia*) were members of the ANT-C. He believed that the lack of overt embryonic homeotic phenotypes of *labial* and *Deformed* was a consequence of the highly derived nature of the maggot head; *labial* and *Deformed* mutations were later shown to cause head transformation in homozygous adult clones. He further argued that the interspersion of *fushi tarazu* and *zerknüllt* among the homeotic genes could not be merely fortuitous.

Off the record, membership of the complex (or indeed the idea that there was more than a chance proximity of developmentally significant genes) was sometimes questioned, but additional work proved KAUFMAN correct. Molecular analysis showed that the homeotic genes (and indeed all other interspersed nonhomeotic genes except the cuticle cluster mentioned below) shared homeoboxes encoding sequence-specific DNA-binding domains important to the function of their proteins as transcription factors. Further, it was realized that a cluster of homeobox genes regulating developmental commitments in an integrated manner is an ancient feature of the Metazoa. In 1987 my colleague DICK BEEMAN mapped the homeotic mutations then extant in the red flour beetle, *Tribolium castaneum*. He found that apparent homologs of both ANT-C and BX-C genes were tightly linked, suggesting that a single homeotic complex (HOM-C) was the ancestral organization among insects. As recently related by MCGINNIS (1994), the homeobox facilitated comparative molecular studies that showed that this complex arose very early in animal evolution and that (with the single recognized exception of Drosophila) its integrity had been maintained over hundreds of millions of years. Until recently, homologs of the nonhomeotic members of the complex had not been detected outside of the Diptera, suggesting that these genes originated as a concomitant of evolutionarily advanced aspects of early development in higher flies. However, a homolog of *fushi tarazu* has now been recognized in Tribolium (BROWN *et al.* 1994), and probable homologs have been described in the brine shrimp and grasshopper (AVEROF and AKAM 1993; DAWES *et al.* 1994), leading to the hypothesis that this and other nonhomeotic members of the complex show a rate of sequence divergence much higher than do homeotic genes. Thus, the question of their origin remains unresolved.

The kind of saturation mutagenesis effort devoted to the Antennapedia complex could not detect all of the functions included (Figure 1C). Screening for zygotic effects failed to identify the maternal effect gene *bicoid* (*bcd*) (FROHNHÖFER and NÜSSLEIN-VOLHARD 1987), which plays a critical role in the establishment of embryonic anterior/posterior polarity. The gene *amalgam* (*ama*) and the

zerknüllt paralog *z2* were discovered molecularly, but mutant effects have not yet been described. Perhaps most enigmatic is a molecularly ascertained cluster of genes putatively affecting cuticle synthesis and structurally unrelated to any other members of the complex.

Saturation mutagenesis also proved very important to our understanding of the BX-C. Independent work by three groups detected only three lethal complementation groups (SÁNCHEZ-HERRERO *et al.* 1985; TIONG *et al.* 1985; KARCH *et al.* 1985). Molecular studies showed that they correspond to the only protein-coding transcription units in the complex and that many mutations giving adult transformations affect complex *cis*-regulatory regions. Earlier views that the BX-C (and some ANT-C) genes function within segmental domains were also modified by the discovery that mutant phenotypes correspond to metameric units offset from segments (HAYES *et al.* 1984; STRUHL 1984) and later called parasegments (MARTINEZ-ARIAS and LAWRENCE 1985).

The facility with which homeo genes can be molecularly cloned and studied (see MCGINNIS 1994) has allowed an investigation of the roles of these developmentally significant genes in phylogenetically diverse animals, as well as insights into the evolution of transcriptional regulatory mechanisms. In the past, some of my colleagues have questioned how the arcane results from Drosophila studies could possibly be relevant to more important matters such as mammalian development. Thus, it is especially satisfying to contemplate how the genetic and molecular characterization of these bizarre mutations has led to the discovery of a gene complex that is providing ever increasing insight into mammalian regulatory mechanisms and incidentally has been proposed as the criterion that defines members of the animal kingdom (SLACK *et al.* 1993).

Many thanks to THOM KAUFMAN, BARBARA WAKIMOTO and RICKI LEWIS for helpful suggestions, as well as to MONICA JUSTICE and MARNETTE DENELL for comments on the manuscript.

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