Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

INTRAGENIC RECOMBINATION IN DROSOPHILA: THE *rosy* LOCUS

TWENTY-FIVE years ago in GENETICS, my colleagues and I reported, in two papers, results of experiments demonstrating that intragenic recombination occurs in *Drosophila melanogaster,* a representative higher eukaryote. The first paper (CHOVNICK *et al.* 1964) described genetic fine-structure mapping experiments involving an array of fully viable, XDH^-, rosy eye color mutations representing a single complementation group. Utilizing a selective system to facilitate large-scale sampling, the resolving power of such recombination permitted the elaboration of a linear order of sites within a single gene, quite comparable to that seen in prokaryotes and fungi. The second paper (SCHALET, KERNAGHAN and CHOVNICK 1964) represented an essential "appendix" to the first. Therein we described genetic analysis of an array of mutations induced in the *rosy* region of chromosome *3* and demonstrated that there was only one gene in this region concerned with xanthine dehydrogenase. While the homozygous viable rosy eye color mutations were limited to that gene, the lethal effects seen with certain of the *rosy* mutations were shown to be associated with adjacent vital genes that are functionally and spatially distinct from the *rosy* locus. All of the lethal *rosy* mutations were deletions extending into these vital genes to varying extents.

These papers completed the initial stage of an odyssey that began in my first year in graduate school. I had entered graduate study committed to the pursuit of mechanisms underlying the tissue-specific and temporal control of gene action in development. However, I was diverted from this path in my first year as a graduate student when I read reports on recombination between mutations of the *lozenge* locus (OLIVER 1940; GREEN and GREEN 1949) and other similar genes (see review by LEWIS 1951). These studies involved several different genes, each having multiple mutant alleles, which were subjected to recombination analyses resulting in the recovery of rare recombinants between the mutant alleles. Although invented for use in a broader context (see MCCLINTOCK 1944), the term *pseudoalleles* came to be used to describe mutations that formerly were considered to be allelic but which subsequently yielded to recombination, and pseudoallelic genes or *complex loci* were expressions used to describe genes whose mutant alleles could be separated by recombination. The use of these expressions reflects the classical conceptual framework within which these cases were interpreted. The classical gene was a unit of function and mutation within which there was no recombination. Recombination occurred only between genes. Hence, the observation of recombination between alleles led to their reclassification as pseudoalleles, members of two separate genetic units in close proximity. Moreover, since mutation of these genetic units produced a similar array of phenotypic effects, it was inferred that these genes were functionally similar or related. An accessory hypothesis (LEWIS 1951) suggested that pseudoallelic genes were instances of gene duplicates in various stages of evolution, and the investigation of such systems was considered to be examining the evolution of new genes and new gene functions. In essence, these studies were interpreted in a fashion entirely consistent with classical notions concerning gene organization.

While LEWIS presented a most persuasive argument for his interpretation of the *bithorax* complex as a cluster of functionally related genes, the interpretation of *lozenge* and other multiply allelic genes on this model seemed open to alternatives. Essentially, multiple functions were inferred purely on the basis of recombination data. In support of the pseudoallelism model was the fact that the mutations fell into a small number of clusters within which recombination seemed not to occur. Yet all of the *lozenge* mutations were recessive, and mutant heteroallelic genotypes were also mutant in phenotype.

These works led me to question the validity of a key feature of the dogma of classical genetics, namely that recombination occurred only between genes and not within a gene. For me, the critical experiment was to examine a single gene with a single, simple mutant effect and with many mutant alleles exhibiting no

evidence of complementation or functional complexity. The basic idea was to carry out a recombination study on a scale large enough to identify many separable sites: too many to permit the interpretation of multiple genes and multiple functions. I was convinced that the small number of gene clusters seen, for example, in the *lozenge* case was merely a reflection of inadequate sampling. My Ph.D. mentor, ALLEN FOX, disagreed with this notion, and moreover felt that such a large-scale undertaking would not make a suitable thesis project. Rather, I was directed to a phenotypic analysis of the *lozenge* mutants in the hope that functional distinction among these pseudoalleles would be forthcoming. Such was not the outcome (CHOVNICK and FOX 1953; CHOVNICK and LEFKOWITZ 1956; CHOVNICK, LEFKOWITZ and FOX 1956). Rather, the conclusion of these studies was that these mutations were alleles of a single functional unit.

During the winter of 1953–1954, on my first faculty appointment, I started to collect mutant strains and to construct appropriately marked chromosomes to carry out fine-structure recombination mapping of the *garnet* locus (*g*: 1-44.4), known by a multiple allelic array of noncomplementing eye color mutations. Within a year the first successful intragenic recombinants were recovered. In the absence of a selective procedure, progress was slow. Additionally, these early results were confounded by the recovery of convertants in addition to the crossover products (CHOVNICK 1958, 1961) [see also HEXTER (1958) and CARLSON (1959)]. Although convertants were not seen in prior recombination studies in Drosophila, they were reported in similar studies in fungi (MITCHELL 1955; PRITCHARD 1955) and eventually came to be recognized as an important feature of intragenic recombination (*e.g.*, WHITEHOUSE 1963; HOLLIDAY 1964). Indeed, conversion continues to play a key role in current thinking about recombination mechanisms (*e.g.*, BORTS and HABER 1989; CURTIS *et al.* 1989).

By the fall of 1957, when I was writing the first *garnet* recombination paper (CHOVNICK 1958), I was already contemplating the limitations of my experimental system and the possible alternatives. Several factors were clear to me at this point, not the least of which was that the issue of intragenic recombination was no longer in doubt, at least in prokaryotes and fungi (BENZER 1955; PRITCHARD 1955). I was convinced that the *garnet* work demonstrated that intragenic recombination also occurred in Drosophila. However, this viewpoint was not generally shared with my colleagues in Drosophila research. Given the limited recombination data with only three sites identified at best, and the conversion-like products that looked suspiciously like mutations, additional work seemed necessary to establish this point. Moreover, the origin of the aberrant segregants (*i.e.*, the conversion-like products that resembled reverse mutations) became an issue of considerable interest. I believed that they were recombinational in origin, reflecting extremely tight intragenic mapping (CHOVNICK 1958). A selection system that would permit sampling approaching the scale routinely used in microbial systems was essential for the continued pursuit of these problems. In this context, I began to consider gene-enzyme systems associated with a visible mutant phenotype in the hope that a nutritional selective procedure might be developed. Very few possibilities existed in Drosophila at that time. The report of FORREST, GLASSMAN and MITCHELL (1956) concerning xanthine dehydrogenase and the *rosy* and *maroon-like* genes and the clear evidence of differential gene expression of *rosy* (see review, HADORN 1956) attracted my attention.

In January 1959, I moved to Cold Spring Harbor as Assistant Director of the Biological Laboratory. In this new role I was preoccupied with mastering many administrative responsibilities. Reestablishing a functioning laboratory to continue the *garnet* locus analysis was accomplished only slowly. During this period in 1959, two events provided further encouragement for the xanthine dehydrogenase system. The first was the GLASSMAN and MITCHELL (1959) paper on XDH and the second was a conversation with INGE RASMUSSEN, a postdoctoral fellow in ED LEWIS' laboratory who attended the Cold Spring Harbor Symposium that year. From RASMUSSEN I learned of the construction of compound autosome arm strains in LEWIS' laboratory. Thus, I knew that half-tetrad analysis, essential for the study of conversion, would be possible with the *rosy* locus on chromosome *3*.

Somewhat later, in the fall of 1959, ABE SCHALET joined my research staff. This provided an opportunity to take a new research direction. We initiated work with both *rosy* and *maroon-like*. However, the complementation seen with our first *maroon-like* mutations and the maternal effect associated with *maroon-like* were unfathomed complexities that led me to favor *rosy* as our initial gene of interest. (But we did return to *maroon-like* later: see FINNERTY 1976.) Very early in conversation with SCHALET I emphasized the importance of a selective system for the recombination work. Shortly thereafter, he proposed the flanking-lethal crossover-selector idea that served as the basis for our early fine-structure mapping studies. A pilot experiment mapping the two original rosy mutations (ry^1 and ry^2) was successful (SCHALET and CHOVNICK 1960). We then dropped all work on the *garnet* locus in order to mount a factory-like operation on *rosy*. At some point prior to our first reports on the *rosy* locus work, SCHALET stumbled over the WHITTINGHILL (1950) paper which describes lethal-crossover selector systems for the study of radiation-induced crossing

over in Drosophila males. At that time, he and I agreed that we simply would acknowledge WHITTINGHILL's priority (SCHALET and CHOVNICK 1960; CHOVNICK *et al.* 1962). The use of the crossover-selector scheme has now come full circle in that we have made use of it in our recent studies of *P*-element transposase-induced male recombination (MCCARRON *et al.* 1989; DUTTAROY *et al.* 1990).

With this beginning, subsequent genetic analysis of *rosy* has focused largely on two topics: gene conversion and its relationship to recombination mechanisms, and gene organization and expression.

In all of the early work on *rosy*, utilizing the lethal-selector system applied to random-strand mapping, conversion products not associated with a single crossover between the flanking lethals were killed. This simplified interpretation of the mapping data and made for easy acceptance of the notion of intragenic crossing over in higher eukaryotes. In 1963 my attention returned to gene conversion when JOHN LUCCHESI mentioned in conversation that *deep orange* (*dor*: 1-0.3) and *rosy* were lethal in double-mutant zygotes (see LUCCHESI 1968). This fact, coupled with the availability of *C(3)L;C(3)R* strains developed by LEWIS (reviewed in HOLM 1976), led me to design a selective system for the study of conversion in half-tetrads utilizing *dor;ry* as a synthetic-lethal combination. DAVID HOLM started graduate work in the spring of 1964 and spent more than a year constructing strains for that first conversion experiment. A cumbersome but effective scheme was constructed and successfully used in one experiment but was never published. Then, in a review article, GLASSMAN (1965) mentioned the discovery of purine selection against XDH^- flies, citing a manuscript in preparation. To my knowledge, nothing further on this topic appeared from GLASSMAN's laboratory. However, we seized upon his suggestion and developed the purine selection schemes that were used in all of our subsequent work with both *rosy* and *maroon-like*. The recombination work, entirely consistent with fungal studies, has been reviewed in recent years (HILLIKER and CHOVNICK 1981; HILLIKER, CLARK and CHOVNICK 1988).

The second direction of our genetic studies dealing with gene organization and expression was stimulated by FRANCIS CRICK's "General Model for the Chromosomes of Higher Organisms" (1971). The model was an attempt to relate the huge excess of DNA found in higher organisms relative to prokaryotes to some features of higher-organism chromosomes. Sometime prior to the appearance of this paper, I received a preprint accompanied by a question from CRICK about the location of our *rosy* mutations. Was I able to position them in either a polytene band or interband region? In the context of his paper, the question really asked if I could locate the mutant sites as lesions of a control or coding region of the gene. The model proposed that the bulk of the DNA (located in the polytene bands) served control functions, in contrast to peptide-coding DNA localized to the interbands. In fact, I had no information other than a linear order of mutations on a genetic map, and some simple phenotypic data. This question served to add a new dimension to our research strategy. With the help of MARGARET MCCARRON, joined shortly thereafter by BILL GELBART and JANARDAN PANDEY, a genetic outline of the organization of the *rosy* locus was developed that offered no evidence for a huge excess of control DNA in contrast to the coding DNA (reviewed in CHOVNICK, GELBART and MCCARRON 1977). This work, supplemented by the efforts of STEVE CLARK, ART HILLIKER and JANIS O'DONNELL (see CHOVNICK *et al.* 1978; HILLIKER *et al.* 1980) attracted the attention of several groups of molecular biologists who tried to clone the *rosy* gene using various state-of-the-art strategies of that time.

The successful cloning of the *rosy* region DNA by BENDER, SPIERER and HOGNESS (1983) and the precise localization of the *rosy* DNA (CLARK *et al.* 1986; COTÉ *et al.* 1986) were factors in the choice of *rosy* for the first *P*-element-mediated transformation experiments (RUBIN and SPRADLING 1982). Our collaboration with WELCOME BENDER and his staff has had a major impact upon the work of my laboratory in recent years (reviewed in DUTTON and CHOVNICK 1988; see also REAUME, CLARK and CHOVNICK 1989). Although somewhat broadened in scope, the odyssey continues with our focus upon such basic genetic mechanisms as recombination and gene expression and the impact of position effects and transposable elements upon these mechanisms.

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