Perspectives

Anecdotal, Historical And Critical Commentaries on Genetics

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Worm Spadework

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I fell in love with *Caenorhabditis elegans* in the summer of '72. Our relationship was cemented four years later, 20 years ago now, by the publication of a paper in GENETICS on *C. elegans* chromosome rearrangements (HERMAN *et al.* 1976). My pleasant assignment here is to describe the beginnings of that work and to relate it to current worm cytogenetics and chromosome mechanics.

In 1972 my research experience had been limited to *Escherichia coli* genetics, but I was caught up in the prevailing restless mood of many phage and bacterial geneticists who were thinking about switching to eukaryotes. SYDNEY BRENNER's plans for the worm first became known to me in 1971 from a brief but intriguing news account in *Nature* of a talk he gave to the Royal Society. [For a description of the beginnings of *C. elegans* work, see HODGKIN (1989).] I had been learning genetics in the best possible way, by teaching it, and the idea of starting over on the classical genetics of a model eukaryote was very appealing, so I jumped at the chance to take a three-week summer course on *C. elegans* at Cold Spring Harbor Laboratory in August of 1972 (the only time the course was given). There were eight students, including GÜNTER VON EHRENSTEIN, who became converted to worm research, and CHRIS GUTHRIE, who did not. The instructors were DICK RUSSELL, who had learned about *C. elegans* as a postdoctoral fellow working mostly on phage and bacteria with BRENNER, and RUTH PERTEL, who had been trained as a more traditional nematologist. (Sadly, VON EHRENSTEIN died in 1980, RUSSELL in 1994.) Our teaching assistant was DAVE DUSENBERY, with whom I shared a home town (Vancouver, Washington) and training in biophysics. In course exercises, we induced Dumpy and Uncoordinated mutants with ethyl methanesulfonate, conducted simple crosses (we were unaware of any genetic maps), and did Feulgen staining of nuclei. Apart from the graduation ceremony presided over by MAX DELBRÜCK in what I took to be an archbishop's costume (see SUSMAN 1995), the high point of the three weeks was a mini-symposium on the last day, attended by many visitors, including several from the BRENNER lab at the MRC Laboratory of Molecular Biology in Cambridge, England.

I wrote to BRENNER in the fall of '72 proposing to spend a sabbatical furlough in his laboratory and suggested working on extragenic suppressors, genetic mosaics, or mutations affecting gametogenesis. He put me off until 1974–75. Meanwhile, I did a little worm genetics in my lab at Minnesota, while two graduate students continued their *E. coli* work. An important influence at the time was the Drosophila work of JUDD *et al.* (1972), which suggested that it took only about 5000 genes to make a fly, and I vaguely hoped that it might some day be possible to study the cell-by-cell effects of mutations in essential genes in *C. elegans* (something I am now doing, using genetic mosaics). A pilot screen showed that it was fairly easy to identify Sterile mutants, but it quickly became obvious that chromosome balancers, standard equipment in Drosophila genetics, would be very useful. I found pairs of loosely linked mutations that conferred visible phenotypes and ran one unsuccessful screen for X-ray-induced crossover suppressors before going with my family to Cambridge in the fall of 1974.

My timing could not have been luckier. BRENNER's famous 1974 GENETICS paper came out that summer, assigning 300 mutations to 100 genes, mapping them onto six linkage groups, and describing how to do worm hermaphrodite genetics. Also available was JONATHAN HODGKIN's newly completed Ph.D. thesis, which contained useful lore on mutagenesis, suppression, and meiotic *X* chromosome nondisjunction. My goal was to develop some of the genetic tools that had proved so useful in Drosophila genetics. PETER LAWRENCE directed me to some fly literature; he clearly appreciated the genetic tools available to fly workers, but I think he was surprised that someone would want to do spadework.

My first experiment in Cambridge, suggested by BRENNER, yielded a useful chromosome rearrangement, an X-ray-induced duplication of the right end of the *X*

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chromosome that was translocated to chromosome *V*. This was equivalent to a half translocation; the scheme used in identifying it, which involved following the transmission of an unlinked *X* duplication from irradiated father to son, precluded recovery of the other half. Clearly it was BRENNER's new genetic map and the large collection of mapped mutants that made this work possible. I was surprised to find that the duplication, now called *mnDp1(X;V)*, showed no detectable recombination with the homologous region of the *X*. It was also homozygous inviable. These features meant that it could be used rather handily to balance lethal mutations, including a set of overlapping deletions that could be used for rapid complementation mapping of point lethals, in the corresponding region of the *X* chromosome. P. MENEELY was later to use *mnDp1* as a balancer of this sort in his Ph.D. thesis work (MENEELY and HERMAN 1981). Additional balancer chromosomes, involving different kinds of chromosome rearrangement, have since been collected by us and by others (EDGLEY *et al.* 1995). For example, ROSENBLUTH and BAILLIE (1981) discovered that one of BRENNER's original uncoordinated mutants contained a reciprocal translocation that dominantly suppressed recombination over large regions of chromosomes *III* and *V*. Over half of the genome is now covered by balancers, but more good balancers are still needed. A curious feature of balancers, probably related to the nature of chromosome pairing, is that one end of each chromosome seems to be much more susceptible to crossover suppression than the other (ZETKA and ROSE 1995).

None of the next four *X* duplications I recovered seemed to be linked to anything. My suspicion that these might be chromosome fragments or free duplications, unattached to any other chromosome, was confirmed when DONNA ALBERTSON, my Cambridge labmate who had been staining *C. elegans* chromosomes with the fluorescent dye Hoechst 33258, looked at oocytes of animals carrying one of the unlinked duplications: she often saw a small fragment in addition to the normal six bivalents (HERMAN *et al.* 1976).

Cytogenetics: NIGON (1949) had much earlier shown that *C. elegans* hermaphrodites have six pairs of chromosomes (males have five pairs and a single *X*), all small, featureless, and about the same size. BRENNER's six linkage groups corresponded properly to the cytological chromosome number, but it was impossible to assign a particular linkage group to a particular chromosome. It was nice to be able to see the small free duplications cytologically, but the only benefit was in confirming that they were free. Some other rearrangements—insertional translocations and asymmetric reciprocal translocations—have also resulted in distinctive karyotypes, but deletions, inversions, and many other translocations have not. *C. elegans* cytogenetics at this stage was obviously primitive compared with the cytogenetics of Drosophila. What finally cured our envy of polytene chromosomes was the development by JOHN SULSTON, ALAN COULSON and colleagues of the *C. elegans* physical map, consisting of overlapping cosmid and YAC clones of genomic DNA [reviewed by COULSON *et al.* (1995)]. Cytogenetics helped reciprocally in the early development of the physical map: ALBERTSON (1985) used cytologically detectable chromosome rearrangements to map cosmid clones to particular chromosomal regions within about 20% of a chromosome length, by *in situ* hybridization and fluorescence microscopy (FISH). Now that the physical map is essentially complete, cloned DNA is positioned on the map more directly. Indeed, a filter spotted with a grid of worm DNA cloned as yeast artificial chromosomes and selected for coverage of the genome is called a "polytene" filter because it is used in the same way that Drosophila polytene chromosomes are used to map cloned DNA by *in situ* hybridization.

The physical map has made it possible to do *C. elegans* cytogenetics at high resolution: ALBERTSON (1993) has used FISH to locate chromosome rearrangement breakpoints within specific cosmids on the physical map. She has also used FISH to localize specific interphase chromosomes by "painting" (CHUANG *et al.* 1994; ALBERTSON *et al.* 1995) and to study meiotic chromosome pairing and segregation (see below).

Centromeres: The free duplications described in our 1976 paper seemed to segregate faithfully during most mitotic divisions, as if they had centromeric function. Either we were lucky to pick a region that contained the *X* centromere or the *X* does not have a single localized centromere. Centromeric constrictions were not apparent cytologically, and free duplications of other regions of the *X* were later obtained. It had been suggested earlier that certain nematodes have diffuse centromeres (TRIANTAPHYLLOU 1971), as do certain other animals, plants, and protozoa (WHITE 1973). Some members of the nematode family Ascaridae had long been known to have atypical centromeres. BOVERI showed over 100 years ago that in the somatic cells of *Parascaris equorum*, the ends of each chromosome are cast off into the cytoplasm, where they ultimately degenerate, and the central segments split into many small chromosomes, each of which retains centromere function; at the same time, the large unfragmented germline chromosomes appear to have multiple spindle attachment points (WHITE 1973). *C. elegans* chromosomes do not undergo chromatin diminution and fragmentation (EMMONS 1988), but ALBERTSON and THOMSON (1982) showed by serial section microscopy that they are holocentric: the microtubules of the mitotic spindle attach to kinetochores that extend along the entire lengths of the condensed chromosomes (each of which is only about 1–2 μm long). It is unclear whether kinetochore formation requires specific centromeric DNA sequences sprinkled along the lengths of the chromosomes. The fact that DNA of apparently any sequence injected into the hermaphrodite gonad forms extrachromosomal arrays that behave much like free duplications cytologically and genetically

(STINCHCOMB *et al.* 1985) suggests that specific centromeric sequences may be unnecessary. On the other hand, differences in mitotic stability among various free duplications and extrachromosomal arrays (see below) suggest that centromere function may be affected by *cis*-acting DNA sequences.

Meiotic centromeres seem wholly unrelated to the mitotic ones. No meiotic kinetochores are apparent in electron micrographs: the spindle microtubules appear to project directly into the chromatin (ALBERTSON and THOMSON 1993). The meiotic chromosomes also orient differently on the metaphase plate. The chromatids of the meiotic bivalent are held together in an end-to-end association, which may be generated by the terminalization of chiasmata. ALBERTSON and THOMSON (1993) showed by FISH that for any given bivalent, the ends that are associated can be either the left or right ones as defined by the genetic map. Furthermore, each bivalent appears to orient with its axis perpendicular to the metaphase I plate; the attached ends are on the plate prior to disjunction, with the opposite ends of each pair of sister chromatids pointing toward the spindle poles. The spindle pole proximal ends then provide the centromeric function of leading the way to the poles at anaphase I. They also appear to keep sister chromatids attached until disjunction at anaphase II (just as classical centromeres do), at which time the opposite ends of the chromatids appear to lead the way to the poles (ALBERTSON and THOMSON 1993; D. ALBERTSON, personal communication). Thus, it seems that at meiosis I either end of a chromosome may act as the centromere, and at meiosis II the opposite end is the centromere! The chromosomes are very compact at both meiotic anaphases, however, so that the chromosome "end" centromeres do not seem very localized and might be defined simply by their proximity to the spindle poles. The molecular mechanisms of meiotic centromere function are obviously quite mysterious.

Free duplications and genetic mosaics: We presented suggestive evidence in the 1976 paper that free duplications were subject to loss during the premeiotic divisions of the hermaphrodite germline, and we mentioned the possibility of using the somatic loss of free duplications to generate genetic mosaics. But the only genetic markers at that time known to be covered by free duplications were loci that conferred an overall uncoordinated phenotype, and we couldn't see how to go about using them to identify mosaic worms. Our inspiration for wanting to generate mosaics of course came from Drosophila: the power of mosaic analysis had been amply demonstrated earlier in flies (for example, STERN 1968; HOTTA and BENZER 1972).

I returned to this problem on my second sabbatical furlough to Cambridge, in 1981–82. By then, additional markers were known. Particularly useful were mutations that ED HEDGECOCK had shown affected the uptake in living animals of the fluorescent dye FITC by groups of chemosensory neurons in the head and tail (PERKINS *et al.* 1986). By using a combination of genetic markers, I showed that the spontaneous somatic loss of free duplications carrying wild-type alleles of genes that were otherwise homozygous mutant did generate genetic mosaics and that such mosaics yielded information about the cell or tissue specificity of gene function (HERMAN 1984). Crucial to the interpretation of these experiments and all subsequent mosaic analyses was the essentially invariant and completely known cell lineage (SULSTON *et al.* 1983), which made it possible to figure out where in the lineage a duplication was lost. I was proud of one experimental design in which somatic loss at a specific cell division of a duplication carrying two visible markers resulted in a phenotypic recombinant, which could be readily identified among the many nonmosaic siblings. For this design to work, the foci of action of the two genes must be different. If, for example, gene a^+ is needed in motor neurons and gene b^+ is needed in body muscle, then a mosaic in which the a^+b^+ duplication is present in motor neurons and absent in muscle would be phenotypically wild-type with respect to *a* and mutant with respect to *b*. Mosaicism in the animal could then be confirmed by scoring the dye-filling phenotype conferred by a third marker, and transmission of the duplication to the germline could also be monitored by scoring self progeny. When I described this scheme to HEDGECOCK, he wondered why it had taken me so long to come up with it. Of course, the trick was in finding the markers that made it work; one of the critical ones came from GREENWALD and HORVITZ (1980).

In 1985 an abstract by HEDGECOCK that appeared in *The Worm Breeder's Gazette* gave mosaic analysis a big boost. *The Worm Breeder's Gazette* was founded by BOB EDGAR in 1975 and has been used by *C. elegans* workers ever since to communicate preliminary findings, work in progress, and other news about *C. elegans* to everyone else in the field. HEDGECOCK reported that a mutation in the gene he called *ncl-1* results in enlarged nucleoli, is cell autonomous, and makes it possible to score, by Nomarski microscopy, nearly every cell in a living animal for the presence or absence of an *ncl-1(+)*-bearing duplication. I learned to score *ncl-1* mosaics during my third sabbatical leave, 1989–90, in HEDGECOCK's laboratory at Johns Hopkins. *ncl-1* has been used as a duplication marker in the mosaic analysis of many genes [reviewed by HERMAN (1995)]. It has also been useful in clarifying the nature of spontaneous somatic duplication loss (HEDGECOCK and HERMAN 1995); for example, most patterns of mosaicism can be traced to duplication loss by a single cell—which often involves nondisjunction, with the sister cell receiving two copies—but occasionally a duplication is transmitted to only a single daughter cell for two or three consecutive cell divisions (a temporary pattern of linear inheritance), after which it recovers and is transmitted to all remaining progeny cells.

Additional advances in mosaic analysis extended

the use of *ncl-1* as a cell-autonomous marker to the study of genes that are not normally present on a *ncl-1(+)*-containing free duplication. For example, a *ncl-1(+)*-bearing duplication can be fused either to an unlinked free duplication (HUNTER and WOOD 1992; HEDGECOCK and HERMAN 1995) or to an extrachromosomal array of cloned DNA carrying a gene of interest (LEUNG-HAGESTEIJN *et al.* 1992). Finally, in what will probably become the most popular technique of all, an extrachromosomal array containing *ncl-1(+)*, at least one visible marker, and the gene to be analyzed can be generated by germline transformation and used as a kind of synthetic free duplication, which yields mosaic animals by somatic extrachromosomal loss (LACKNER *et al.* 1994; L. MILLER, D. WARING and S. KIM, personal communication).

Different free duplications can exhibit very different frequencies of spontaneous mitotic loss (HEDGECOCK and HERMAN 1995). Duplication size affects mitotic stability, as does a chromosomal mutation that affects chromosomal segregation, but other factors affecting duplication stability—and perhaps, concomitantly, normal chromosome stability—also seem to be important, but remain to be elucidated. Finally, we do not understand why free duplications acquire deletions at very high rates ($>10^{-3}$ per generation) during germline transmission.

Conclusion: *C. elegans* cytogenetics and chromosome mechanics have turned up unexpected and interesting findings concerning the behavior of holocentric chromosomes and free duplications. But I think the most important contribution of work in this area has been to facilitate the analysis of developmental and behavioral mutants. The wonderful progress that the *C. elegans* field as a whole has enjoyed, which has relied heavily on mutant analysis, has amply justified the genetic spadework that has been done—and should justify continued spadework.

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