THE DISCOVERY AND SIGNIFICANCE OF MOBILE GENETIC ELEMENTS

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1. Introduction

One of the most intriguing facts about the history of mobile genetic elements is that no one set out to discover them. In every case, they were initially found by geneticists studying other problems: chromosome breakage and rejoining in maize, transfer of genetic information between bacterial cells, lysogeny, regulation of gene expression, antibiotic resistance. Is there a message in this repetitive serendipity? I think so. The message appears to concern the power that unspoken assumptions hold over our thinking (in this case, the idea that the genetic material is basically stable) and the need to be alert for those unexpected findings which overturn those assumptions and suddenly open a whole new universe for scientific exploration. Such major reorientations in thinking are inevitable in science (39).

Genetics has travelled a long conceptual road from the Constant Genome paradigm of the 1940s, when McClintock first described transposable controlling elements in maize, to the Fluid Genome paradigm that reigns today. There is an even longer and more fascinating voyage ahead as we come to terms with the implications of mobile genetic elements and molecular discoveries about genome organization and reorganization. This historical chapter affords a rare opportunity to trace the outlines of the intellectual journey already completed as well as to attempt some projections of where we might be going. The emphasis here will be on new ways of looking at genetic problems, on key observations, and on the unexpected convergence of discoveries in separate experimental systems. Accounts of the history of particular systems with a more mechanistic focus can be found in the first *Mobile Genetic Elements* book (62).

2. Maize controlling elements

Barbara McClintock discovered transposable elements in maize after many years of studying chromosome breakage and its genetic consequences (54). Her work on chromosome breakage began by investigating genetic instabilities associated with X ray-induced chromosome rearrangements, especially ring chromosomes (44). She had found that nuclei harboring two broken chromosome ends will join them together quite rapidly and efficiently. Depending upon the positions of the breaks, these joining events created a wide variety of chromosome abnormalities, including translocations, inversions, duplications, deficiencies (what we now call deletions) and chromosome losses. McClintock had learned to follow the loss of specific markers as chromosome changes occured during development by observing variegated patterns of pigmentation in maize plants and kernels.

In the early 1940s, McClintock set out to investigate the genetic content of a single chromosome arm. She had devised a system to initiate breakage in the short arm of chromosome 9 during meiosis. Self-fertilization of plants carrying these broken chromosomes would lead to the formation of embryos undergoing what McClintock termed the chromosomal type of breakage-fusion-bridge cycle (see the papers in reference 54 for a full account). Plants emerging from zygotes subjected to the breakage-fusion-bridge cycle were expected to contain modified chromosome 9s, and they did. But they also displayed new kinds of genetic instability, which McClintock had not observed before. Instead of the sporadic patterns of chromosome loss she was familiar with from her breakage studies, the new instabilities showed certain regularities which told her that a controlled process was taking place. She spent the next three decades documenting the genetic elements which underlay these instabilities and uncovered an unsuspected array of genomic systems capable of changing chromosome structure and altering the regulation of gene expression. Because of their capacity for creating different patterns of gene expression during development, McClintock applied the name "controlling elements" to the newly discovered mobile genetic systems (49).

One of the novel types of genetic instability McClintock found was manifested as repeated breakage events at a specific chromosomal locus. McClintock called these specific breakages

"dissociation" and labelled the responsible element at the breakage site Ds. The breakages could be followed by observing clonal patches of tissue (sectors) in either maize plants or kernels in which dominant markers distal to Ds on the chromosome had been lost. How many such clones were formed and when they arose during plant or kernel development was often quite regular. In other words, the frequency and timing of chromosome breaks was non-random and under some kind of control. The control system could be reset, however, and when this resetting happened, sectors or plants would appear in which a new frequency/timing regime was expressed. The Ds system was also quite regular in that the same group of markers was lost, and physical breaks could be seen through the microscope to occur at the appropriate position in chromosome preparations (46). Occasionally, however, a plant sector or kernel was observed to change pattern such that a new group of markers was lost in each sectoring event. Such results indicated that Ds had changed its position, and this new position could be confirmed by cytological examination. Thus, Ds was found to be capable of transposition from one site to another.

Genetic crosses quickly revealed that regulated chromosome breakage did not depend only on the Ds element at the site of breakage events. Chromosomes carrying Ds could be quite stable in some plants, but they would again show breakage when introduced into plants that carried another genetic element, termed Ac for activator of Ds. Like Ds, Ac could transpose from one location to another. The timing and frequency of Ds breakage events was also regulated by Ac: the higher the Ac dosage in a tissue, the later Ds breaks occured during development. This Ac-Ds system came to be the prototype for what are now called "two element systems," where the activity of one dependent transposable element is conditional upon functions encoded by another autnonomous element. Other examples of two-element systems include the Dotted (Dt) and Supressor-Mutator (Spm) autonomous elements, each of which has its corresponding family of dependent elements. Ac does not activate Spm- or Dt-responsive elements, and neither Spm nor Dt will induce genetic instability at Ds. These maize systems were, incidentally, the first examples demonstrating that specific gene products encoded by one locus could regulate the behavior of another locus, and McClintock drew attention to some parallels of controlling element functions with the kinds of regulatory molecules described by Jacob and Monod (50).

The second novel type of genetic instability coming from the self-fertilized breakage-fusion-bridge cycle plants involved "mutable loci" (45). These were mutant loci affecting some visible phenotype, such as pigmentation, which gave rise to variegation patterns showing changes from the recessive to the dominant phenotype during development. For example, clonal patches of pigmented cells would appear on a mutant, unpigmented aleurone layer of a kernel. These mutable loci could affect many different characters, such as chlorophyll and anthocyanin pigmentation or endosperm starch synthesis, and mutable alleles could be found at any known locus where a suitable selection scheme was applicable (47). Superficially, the recessive to dominant variegation at mutable loci was the opposite of the patterns given by chromosome breakage, where marker loss gave a change from dominant to recessive phenotype. But the underlying similarity between genetic events at Ds and at mutable loci became manifest when it was found that the presence of Ac was necessary for instability to appear at a number of mutable loci. Ultimately, it became clear that some mutable alleles represented transpositions of Ds elements into particular loci and that recovery of dominant expression was the result of Ac-dependent Ds excision from the locus. Other mutable alleles resulted from the insertion of Ac itself or of controlling elements belonging to the Spm (= suppressor-mutator) or other controlling element families (51).

One feature of mutable alleles which needs to be mentioned is the variety of expression patterns they generated at any given locus. Variegation was only one kind of novel pattern. Quantitative changes in stable expression could be observed in the absence of the mutator (transposase) function of the autonomous element, and a number of mutable alleles gave rise to

different levels of expression following element excision (51). Other changes affected tissue specificity and spatial distribution of expression in the absence of variegation. McClintock derived variants of anthocyanin pigmentation loci which encoded kernel patterns similar to those treasured by Amerindians for religious purposes (51). In addition, she has indicated that controlling elements can be used to analyze the genetic basis of nonclonal patterns, where groups of cells that are not related to each other by descent display common phenotypes (52; 66).

After a decade of intensive genetic and molecular analysis, we are beginning to have a detailed picture of how controlling elements move through the maize genome and of the myriad mechanisms by which they alter the expression of information encoded at specific loci (21). Even without the molecular details, however, McClintock's discoveries had revolutionary implications. First, of course, was overturning the Constant Genome notion. Her work showed that cells contained activatable systems that could restructure the genome. Once recombinant DNA methods made all organisms amenable to molecular genetic analysis, the universality of mobile genetic elements and the diversity of their molecular mechanisms became clear. Today we think of the Fluid Genome.

A second fundamental consequence of McClintock's work concerned the concept of the unitary gene. If controlling elements could insert into individual loci and alter their pattern of expression during development, then each genetic locus was not occupied by a series of indivisible alternate alleles. Instead, a genetic locus is a complex mosaic structure that can be modified by the addition and removal of specific genetic elements. A related third point to emerge from McClintock's work was the realization that individual loci are not autonomous units but can be connected into coordinately controlled systems. When members of the same controlling element family inserted into two loci, expression of those hitherto separate loci came under joint control of the cognate autonomous element. Simple examples like these illustrated how repetitive genetic elements could be distributed to many sites in the genome to constitute the physical basis for an intranuclear regulatory network (48).

3. Plasmids, phages and episomes

The second chapter in the history of mobile genetic elements began in the late 1940s with the effort to understand two of the mechanisms of genetic exchange in bacteria: cell-to-cell conjugation and bacteriophage-mediated transduction. The serendipity theme has been very strong in bacterial genetics. Bill Hayes discovered the F plasmid as part of a long-term effort to understand phase variation. Elie Wollman and Franç ois Jacob discovered F insertion in the circular bacterial chromosome as a consequence of experiments designed to map the λ prophage. Alan Campbell figured out reciprocal recombination in prophage insertion and excision as the consequence of mapping defective λ transducing phages. No one anticipated that the concept of episomes (mobile genetic elements which can exist in two alternative states, either autonomous or attached to the bacterial chromosome) would grow out of studying the distinct phenomena of genetic recombination and lysogeny.

Hayes' experiments were technically simple but conceptually elegant. Following the discovery of *E. coli* recombination by Lederberg and Tatutm (41), Hayes used minimal medium to detect the formation of recombinants between auxotrophic mutants. His innovation was to use streptomycin as a selective agent in his crosses (27). Because streptomycin kills potential recombinants before they can form colonies, Hayes could distinguish the bacteria in a fertile cross into donors and recipients. Streptomycin-sensitive donors and streptomycin-resistant recipients would form recombinant colonies on medium containing streptomycin because the recombination events took place in a resistant cell. Streptomycin-resistant donors and streptomycin-sensitive recipients, on the other hand, would not form recombinant colonies on streptomycin medium because the antibiotic would kill the cells where recombination events

were occuring. Armed with this test to identify donor bacteria, Hayes discovered that they readily conferred donor ability on recipient cells and that the donor character spread through a recipient population much faster than individual cells could divide (28). He concluded (i) that there must exist an infectious fertility factor, F, whose presence permitted F^+ donor *E. coli* cells to transfer DNA to F^- recipient cells and (ii) that F must replicate independently of and faster than the bacterial chromosome. In this way, he defined the first bacterial plasmid and opened the door to a rigorous analysis of bacterial sexuality.

Hayes also found that donor cells only transferred a segment of their genomes to the recipient cells. Elie Wollman and Franç ois Jacob answered how the F plasmid mediated this partial transfer through their analysis of more potent donor strains. These were termed Hfr for high frequency of recombination because they could transfer certain markers orders of magnitude more frequently than F^+ donors. By examining a number of different Hfr strains and using a Waring blender to disrupt contacts between donor and recipient cells, they demonstrated that each Hfr strain transferred genetic markers in an oriented manner from a fixed point on the circular bacterial chromosome (35, 78). The transfer process took about 90 minutes to encompass the whole chromosome, and most mating pairs fell apart before this time, thus giving the partial transfer found by Hayes. The fixed point where transfer originated was interpreted as a site where the F plasmid inserted into the bacterial chromosome. Since different Hfr's had different origins of transfer, insertion could occur at many locations. The genetic and physical connection between F and the chromosome in Hfr strains was established by the isolation of so-called F' plasmids which replicated autonomously but carried segments of the bacterial chromosome that previously had been located adjacent to the Hfr origin of transfer (1). These F' plasmids represented, in fact, some of the earliest examples of molecular cloning.

An even earlier example of molecular cloning came from studies of transduction by the temperate bacteriophage λ native to *E. coli* K-12. Transduction was first discovered in *Salmonella*, where it was found that some particles of phage P22 could transfer small fragments of the bacterial chromosome from one cell to another (81). P22 mediated a process called *generalized* transduction because it could transfer virtually any marker on the chromosome. When λ was tested for transduction, it behaved differently from P22: λ only transferred fragments carrying the *gal* (galactose utilization) marker, which had been located next to the λ prophage in Hfr crosses (56). λ transduction was called *specialized* transduction because it was limited to certain markers and because it displayed some peculiar features. Transductants received both phage and bacterial markers from the donor strain (57). These markers were incorporated into defective phage derivatives called λ*dgal*. When Campbell analyzed the genetic structure of various λ*dgal* phages, he found that a particular region of phage DNA had always been substituted by a region of bacterial DNA (13), in a way analogous to contemporary cloning in λ vectors. To explain how these recombinant molecules originated, Campbell postulated that prophage insertion involved reciprocal recombination at specific sites between a circular phage molecule and the circular bacterial chromosome. Such an event would generate a larger circle with the (pro)phage DNA continuous with the chromosome at a specific position. Excision would occur by a complementary recombination event. Normally this excisive recombination would take place at the same sites as insertion, but occasionally excision would occur at other sites to yield λ*dgal* derivatives. These recombination steps comprised the well-known "Campbell model" which explained specialized transduction, F insertion into the chromosome to form Hfr's, and aberrant F excision to generate F' plasmids (14). Thus, even though they had many quite distinct properties, both F and λ were classified together as episomes because they displayed similar kinds of interactions with the bacterial chromosome (35). Further work on λ has greatly extended the Campbell model and identified special site-specific recombination enzymes which,

together with their cognate specific DNA substrates, serve as the molecular tools for insertion and excision (40).

The DNA manipulation potential of F' plasmids and specialized transducing phages was quickly appreciated among bacterial geneticists, and *in vivo* genetic engineering became a key tool in analyzing genome structure and function starting in the middle to late 1960's (61). For example, temperature-sensitive replication mutants of F*lac* plasmids were isolated, and the ability of an F_{ts}*lac* plasmid to rescue itself at the non-permissive temperature by insertion into the bacterial chromosome was exploited to transpose the *E. coli lac* operon to new genomic locations (34). Combined with specialized transduction of the transposed *lac* sequences (6), such methods permitted the isolation and purification of defined DNA sequences for molecular analysis even before restriction enzymes and all our other current genetic engineering tools were available (61, 68).

4. Transposable elements in bacteria

The parallels of bacterial episomes to maize controlling elements were only dimly appreciated in the late 1950's and early 1960's. The main reason that the relationship between them was not more widely recognized was the deep conviction that genetic rearrangements were aberrations. From this point of view, each example of genetic mobility was a peculiar exception to normal function, and the underlying molecular processes were stigmatized with the epithet of "illegitimate recombination." Nonetheless, the study of bacterial conjugation and transduction had firmly established in the minds of molecular biologists the concept of genetic elements which could migrate from one location to another through the genome. The use of phages and plasmids for *in vivo* genetic engineering experiments reinforced the appreciation of the capacity for genome plasticity. What finally made it generally evident that mobile genetic elements were an integral part of the genomic landscape was the discovery in bacteria of transposable elements whose only *raison d'etre* seems to be the ability to move DNA segments from one locus to another.

There are three strands to the prokaryotic transposable element story. They all began in the 1960's and came together with two key conferences in the mid 1970's. The first strand began with A.L. Taylor's discovery of a curious bacteriophage in Denver sewage. This phage was called Mu because it had the intriguing property of inserting its prophage at many different sites on the *E. coli* chromosome, sometimes causing mutations (73). Insertions had little sequence specificity. The mutations could occur in virtually any locus on the chromosome, and insertions into the *lac* operon were found at many different positions (11, 17). Since it could be propagated as a phage, Mu was particularly suitable for studying the molecular biology of this genetic promiscuity. Mu DNA could be extracted from purified phage particles, and phage genetics methods could be used to isolate mutants. The molecular studies showed that all aspects of Mu biology were intimately connected with its ability to move through the genome; recombination with different regions of the genome was essential to Mu's ability to replicate and lysogenize (74). Complementary genetic studies of how Mu interacted with different replicons in *E. coli* cells revealed it to be a powerful agent for rearranging the bacterial genome.

The second strand to the bacterial transposable element story was yet another example of multiple serendipity. Bacterial geneticists interested in the regulation of gene expression had isolated spontaneous pleiotropic mutations of the *lac* and *gal* operons (2, 42, 36, 58). These mutations turned out to display unexpected properties (e.g. extreme polarity on the expression of cistrons downstream from the mutant site). They were not deletions because they could revert, but their responses to mutagens in reversion tests were quite different from those of well-known point mutations involving base substitutions or frameshifts. Clearly, these spontaneous mutations represented a new kind of molecular event, and the hypothesis was proposed that they resulted

from the insertion of additional DNA (58). Using specialized transducing phages to analyze the physical structure of the mutations, this hypothesis was quickly confirmed (30, 37, 43, 59), and it was discovered that several distinct segments of DNA could each insert at a number of different sites. Accordingly, these segments were called Insertion Sequences, or IS elements (22, 31). The biological importance of IS elements became even more evident when they were found to be components of bacterial plasmids, such as F and drug resistance determinants, and were located at key positions in those plasmids, including the sites where F recombines with the bacterial chromosome to form Hfr's (18, 32). It was becoming clear that IS elements played a major role in restructuring the bacterial genome through mutation and DNA transfer.

The third strand of the prokaryotic transposable element story involved the study of drug-resistance plasmids. The best-documented contemporary example we have of genetic change in evolution is the emergence of transmissible antibiotic resistance as the bacterial response to antibacterial chemotherapy. Following the discovery of R (resistance) plasmids in Japan in the later 1950s (77), there was intensive research on the genetics of antibitioic resistance determinants. Many of these determinants displayed anomolous recombination behavior and were ultimately found to transpose from one replicon to another (e.g. 29, 38). The term transposon was used to denote these transposable resistance determinants. It later became clear that many other kinds of phenotypic markers could also be carried on transposons, such as the ability to degrade specific substrates or the production of toxins and other virulence factors. Molecular analysis in the 1970s revealed that transposons came in (at least) two broad classes. One class, usually represented by the ampicillin transposon Tn3, had a structure similar to that of IS elements but included one or more coding sequences for a specific phenotypes inside the inverted repeats that marked the element's termini (29, 70). The other class consisted of compound elements, each of which contained a central coding sequence flanked by two copies of a particular IS element (38). Genetic analysis showed that the IS elements conferred genetic mobility on these compound elements and that any region of the bacterial genome could be incorporated into such a composite transposon. Because they played a key role in bacterial survival of the antibiotic onslaught, the biological utility of transposable elements could no longer be questioned.

5. Mobile elements in eukaryotes

The moment when it became clear that episomes and transposable elements were all related is well-defined: it was during a remarkable conference on Bacterial Plasmids held at Squaw Valley in 1975. At that meeting, held shortly after the famous Asilomar Conference on the implications of recombinant DNA, there were reports on IS elements in plasmids, on the genetic activity of phage Mu and on various antibiotic resistance transposons. The idea of genetic engineering was very much in the air, and it was readily appreciated that bacteria were engineering their own genomes. The following year, stimulated directly by the Squaw Valley conference, the first meeting devoted specifically to DNA Insertion Elements, Plasmids and Episomes was held at Cold Spring Harbor Laboratory (11). For the first time, mobile elements in bacteria and in higher organisms were discussed together from the perspective of mobility as a basic feature of all genomes. Genetic data on yeast, *Drosophila*, and animal viruses were invoked to show that the tremendous innate capacity for genome reorganization seen in maize and bacteria were general phenomena. In the years following the Insertion Element meeting, the application of recombinant DNA methods has abundantly verified McClintock's prediction that mutable loci and transposable elements would be found in the genomes of all organisms. The molecular work also revealed an unexpectedly wide variety of biochemical systems for restructuring genomes. The era of the Fluid Genome had arrived.

The chapters that follow will discuss specific examples of genome restructuring in some

detail. Here it is perhaps useful to distinguish eukaryotic mobile genetic elements into two broad categories:

(a) DNA-based mobile elements. All of the mobile elements discussed above in bacteria and maize appear to operate exclusively at the DNA level. The biochemical activities responsible for mobility recognize structural features in the mobile elements, make cleavages both in those elements and at other genomic sites, and then religate the cleaved segments in new ways to create intact but rearranged DNA molecules. Examples of DNA-based elements appear to include the P, Hobo and FB (foldback) elements in *Drosophila*, the Tc1 element of *C. elegans*, and plant elements related to the Ac and Spm systems of maize (see relevant chapters in reference 8). It is important to note that not all DNA-based mobile elements fall into a single mechanistic class. We already know of several different biochemical mechanisms for the movement of DNA-based elements. In bacteria alone, for example, λ uses a reciprocal site-specific recombinase complex (40), Mu and Tn3 have replicative transposition mechanisms (4, 60), Tn7 has a non-replicative cut-and-paste transposition mechanism with a linear intermediate (5), and Tn916 uses a non-replicative excision/reinsertion mechanism with a circular intermediate (15). In other words, genetic mobility operating at the DNA level appear to have arisen several times in evolution with distinct biochemistry.

(b) Retrotransposons and other RNA-based mobile elements. In yeast, *Drosophila* and vertebrates, the most abundant class of mobile elements appears to be those with move through RNA intermediates and utilize reverse transcription to insert at new genomic locations. A major subdivision of these retrotransposons are those which are structurally and mechanistically related to retroviruses, including the Ty elements of yeast, some of the most active *Drosophila* elements including *copia*, 412, B104 and *gypsy* (which are together responsible for the majority of spontaneous visible mutations; 26), and elements in organisms as diverse as plants and slime molds (see relevant chapters in reference 8). It is important to remember that retroviruses are themselves important mobile genetic elements, and their pathogenic properties are often due to insertional mutagenesis or to transduction of modified cellular sequences in the form of viral oncogenes (75). Interestingly, the chromosomal integration of reverse-transcribed retroviral DNA is mechanistically similar to steps in bacteriophage Mu transposition (23, 60). As with the DNA-based elements, there is more than one pathway for RNA-based mobility, and another major group of retrotransposons lack the characteristic terminal repeats of retroviruses. These include the LINEs (long interspersed nucleotide elements; 33), which encode their own reverse transcriptase, and the SINEs (short interspersed nucleotide elements which do not encode reverse transcriptase; 19). In addition, as discussed later in this volume, a new class of reverse-transcribed elements, called retrons, has also recently been discovered in bacteria.

6. Genome reorganization as a biological process

Many mobile genetic elements were initially detected by recombinant DNA analysis as repetitive elements. Molecular studies also revealed another aspect of genomic fluidity in the form of DNA rearrangements that accompany cellular differentiation. These developmental rearrangements involve a wide variety of biochemical mechanisms. For example, both *Salmonella* and *Neisseria* undergo phase variations (on-off switches) affecting the biogenesis of surface structures, but the *Salmonella* system uses a site-specific recombinase (25) while the *Neisseria* system uses homology-dependent recombination (71). In the vertebrate immune system, at least four mechanistically different events are involved in assembling and modifying the sequences which encode specific immunoglobulins during B lymphocyte maturation (3, 9). Some organisms have very regular cycles of genome reorganization during somatic development or gametogenesis. Many of these cycles are lumped together under the rubric of "chromatin dimunition," but that designation includes many mechanistically different events. In some cases,

pieces of chromosomes are excised during development (e.g. 7), while in other cases whole chromosomes are discarded (e.g. 16). Perhaps the most spectacular example of chromatin diminution occurs during macronuclear development in ciliated protozoa (24). In this remarkable process, the entire germ-line genome is fragmented into smaller pieces of DNA, some of these are then joined together in new arrangements, and the thousands of resulting DNA segments are each capped with telomeres to produce mini-chromosomes that direct the synthesis of RNA (80).

What our rapidly expanding knowledge of mobile genetic elements and developmental DNA rearrangements is telling us is that cells have at their disposal a large tool box of biochemical activities for restructuring their genomes. In this context, it is relevant to bear in mind that almost all of the biochemical tools used in human genetic engineering are extracted from one or another cell type. Most of these cellular DNA rearrangement activities are quite sophisticated and involve multiple protein and nucleic acid components organized into intricate dynamic three-dimensional complexes, such as the λ intasome (20, 40) or the retroviral particle (75, 76). Like all biochemical systems, those carrying out DNA rearrangements are subject to cellular and organismal regulatory networks. The ability of these networks to modulate genetic changes in response to developmental or environmental cues is reflected in specific cases, such as the developmental specificity of immune system rearrangements (9) or the starvation-induced activation of a Mu prophage to produce coding sequence fusions that permit the utilization of an alternative growth substrate (12, 50, 63, 69). In other words, the discovery of multiple sophisticated biochemical systems for DNA reorganization means that genetic change is a normal function of living cells (64). There is nothing "illegitimate" about the many nucleases, recombinases, ligases and other rearrangement activities we are uncovering, and their adaptive functions can be studied by the same genetic and biochemical approaches used to elucidate other aspects of cell and organismal biology.

7. Integral view of genome function and evolution

McClintock was quite prophetic when she asserted that mobile genetic elements would profoundly alter our thinking about genome organization and evolution. Not only has the Fluid Genome replaced the Constant Genome, but our thinking about genome structure and function has also undergone a sea-change. McClintock's insight that genetic loci contained distinct, variable regulatory regions has been borne out by numerous studies of the molecular basis of gene expression, especially work on transcriptional regulation and the processing of RNA. Our current conception of the typical genetic locus as a mosaic of exons, introns, 5' and 3' regulatory regions, each composed of its own array of domains and binding sites, is totally different from the classic theory of the unitary gene formed in the pre-DNA era. We now realize that repetitive sequence elements, such as binding sites for transcription factors, are present at multiple unlinked loci and serve as one of the physical bases for coordinately regulated multigenic networks. We also understand that other repetitive elements, such as centromeres and telomeres, play key roles in maintaining the physical organization of the genome.

Comparing this view of the genome as an interactive network of multiple sequence elements with the classical idea of the genome as composed of autonomous genetic units linked together like beads on a string is like comparing our current concept of atomic structure with that of the pre-quantum mechanics era. As we near the end of the 20th century, molecular genetics has led us into a different conceptual universe. It would be strange if such a major change in our thinking about basic genetic organization did not also influence our understanding of evolutionary processes. In order for the integrated mosaic genome to make evolutionary sense, there must exist mechanisms for large-scale, rapid reorganization of diverse sequence elements into new configurations. By analogy with computer-based systems, evolution could be envisaged as involving changes from one system architecture to another (65). When we thought of genetic

change only in terms of point mutations and random events, massive genome reorganization was inconceivable, but the study of mobile elements has now made the inconceivable very real. McClintock (53) observed major genome restructuring in some of the plants emerging from the breakage-fusion-bridge cycle, and we now have many other examples, such as ciliate macronuclear development and multiple pre-meiotic germ-line transpositions in *Drosophila* hybrid dysgenesis (79). As we incorporate our knowledge of DNA rearrangements into basic theories of heredity, it may be useful to think of evolution as a natural genetic engineering process (67). If this viewpoint proves itself intellectually robust enough to direct an effective research agenda, then mobile genetic elements will have moved from the fringes to the center of modern biological thought.

8. Barbara McClintock, 1902 - 1992

This introduction was drafted shortly before the death of Barbara McClintock on September 2, 1992, at the age of 90. Since McClintock was the lone pioneer in the study of mobile genetic elements for so many years and since she has had such a profound impact on modern genetics, it is fitting to add a few words of tribute to her in this introduction. McClintock was one of the outstanding figures in modern science. From the establishment of chromosomes as the physical carriers of hereditary determinants to the analysis of nuclear networks governing gene expression, her 69 year career was an integral part of the genetic revolution which is still transforming our understanding of life.

We are not yet in a position to evaluate the full significance of Barbara McClintock's scientific accomplishments. We recognize that her work on transposable elements revolutionized our thinking about genome stability and genome reorganization. But the implications of her observations that cells can rapidly detect the presence of broken chromosomes and efficiently repair the breaks remain to be fully explored. Likewise, genetic theory has not yet fully incorporated her discovery of repetitive mobile genetic systems that can alter the developmental expression of any genetic locus and can create control networks involving unlinked loci.

The main reasons that McClintock's insights are still outside the mainstream have more to do with attitudes than with data. Even though her thinking was far more sophisticated, a common misconception has persisted that McClintock thought of controlling element insertions and excisions as the chief mechanism of developmental gene regulation. While it is true that excisions did occur in a regulated manner and thus gave rise to developmental patterns, she also documented many novel patterns of gene expression that did not involve mutational events. For McClintock, the main point was that each new controlling element insertion (or change in the structure of a resident element) modified a particular genetic locus and brought it under the control of a wide repertoire of regulatory mechanisms. Similar modifications at two or more loci would create a genetic network. Another obstacle to broader acceptance of McClintock's perspective is the widespread tendency to explain hereditary phenomena in terms of independent genetic units, a holdover from the days when Mendelian segregations were the basic methods of analysis. In contrast, McClintock thought of the genome as a complex unified system exquisitely integrated into the cell and the organism, and her work with controlling elements revealed some of the physical mechanisms that were at the basis of genomic integration.

McClintock's vision extended beyond the genome. One of her most challenging ideas is the concept of "smart cells" (something she used to bring up gingerly in lectures at the end of her career). Behind this concept lay decades of experience. Her own work involved tracing the development of tens of thousands of maize plants in intimate detail, and she was well aware of the work of other scientists, from the 19th century pioneers all the way up to contemporary molecular cell biologists. She was deeply impressed by the ability of cells to sense internal and external cues, evaluate them, and respond with actions appropriate for survival and

morphogenesis. How this monitoring and decision making operate was, she felt, a key area for future exploration.

What made McClintock so special? The answer lies in her complete intellectual freedom. The courage to say, "I do not understand," and the courage to investigate the unexplainable were at the heart of her remarkable success. Many scientists have been upset because Barbara McClintock characterized herself as a mystic. But to her, mystic did not mean someone who mystifies. Instead, for Barbara McClintock, a mystic was someone with a deep awareness of the mysteries posed by natural phenomena. Mystification came, in her view, when we tried to use our current concepts to explain phenomena that demanded new ways of thinking.

Barbara McClintock occupies a unique place in the history of biology. Her work spanned almost the entire 20th century. A main participant in many aspects of this century's revolutionary exploration into the physical basis of heredity, she began her studies only two decades after the rediscovery of Mendelism. Her observations on genetic networks and genome reorganization have defined problems to be addressed in the 21st century. It is possible that McClintock will one day be seen as the key figure marking the transition from the naturalistic biology of the 19th century to the informational biology of the future. Her rich scientific legacy will reward continued study for decades to come.

BIBLIOGRAPHY

1. Adelberg, E.A. and Burns, S.N. (1960) Genetic variation in the sex factor of *Escherichia coli*. *J. Bacteriol.* **79**, 321.
2. Adhya, S. and Shapiro, J.A. (1969) The galactose operon of *E. coli* K-12. I. Structural and pleiotropic mutations of the operon. *Genetics* **62**, 231.
3. Alt, F.W., Blackwell, T.K. & Yancopoulos, G.D. 1987. Development of the primary antibody repertoire. *Science* **238**, 1079.
4. Arthur, A. and Sherratt, D. (1979) Dissection of the transposition process: a transposon-encoded site-specific recombination system. *Mol. Gen. Genet.* **175**, 267.
5. Bainton, R., Gamas, P. and Craig, N. (1991) Tn*7* transposition in vitro proceeds through an excised transposon intermediate generated by staggered breaks in DNA. *Cell* **65**, 805.
6. Beckwith, J.R., Signer, E.R. and Epstein, W. (1966) Transposition of the *lac* region of *E. coli*. *Cold Spr. Harb. Symp. Quant. Biol.* **31**, 393.
7. Beerman, S. 1977. The diminution of heterochromatic chromosomal segments in *Cyclops* (Crustacea, Copepoda). *Chromosoma* **60**, 297.
8. Berg, D.E. and M.M Howe. 1989. *Mobile DNA*. American Society for Microbiology, Washington, D.C.
9. Blackwell, T.K. and Alt, F.W. (1989) Mechanism and developmental program of immunoglobulin gene rearrangement in mammals. *Ann. Rev. Genet.* **23**, 605.
10. Bukhari, A.I. and Zipser, D. (1972) Random insertion of Mu-1 DNA within a single gene. *Nature New Biol.* **236**, 240.
11. Bukhari, A.I., Shapiro, J.A. and Adhya, S.L. (eds.) (1977) *DNA Insertion Elements, Plasmids and Episomes*. Cold Spring Harbor Laboratory.
12. Cairns, J., Overbaugh, J. and Miller, S. (1988) The origin of mutants. *Nature* **335**, 142.
13. Campbell, A. (1959) Ordering of genetic sites in bacteriophage λ by the use of galactose-transducing defective phages.*Virology* **9**, 293.
14. Campbell, A. (1962) Episomes. *Advanc. in Genetics* **11**, 101.
15. Caparon, M.G. and Scott, J.R. (1989) Excision and insertion of the conjugative transposon Tn*916* involves a novel recombination mechanism. *Cell* **59**, 1027.
16. Crouse, H.V. (1960) The controlling element in sex chromosome behavior in *Sciara*. *Genetics* **45**, 519.
17. Daniell, E., Roberts, R. and Abelson, J. (1972) Mutations in the lactose operon caused by bacteriophage Mu. *J. Mol. Biol.* **69**, 1.
18. Davidson, N., Deonier, R.C., Hu, S. and Ohstubo, E. (1975) Electron microscope heteroduplex studies of sequence relations among pladmis of *Eschericha coli*. X. Deoxyribonucleic acid sequence organization of F and of F-primes and the sequences involved in Hfr formation. In *Microbiology 1974*. Schlessinger, D. (ed), American Society for Microbiology, p. 56.
19. Deininger, P.L. (1989) SINES: Short interspersed repeated DNA elements in higher eucaryotes. In *Mobile DNA*. Berg, D.E. and Howe, M.M. (eds), American Society for Microbiology, p. 619
20. Echols, H. (1986) Multiple DNA-protein interactions governing high-precision DNA transactions. *Science* **233**, 1050.
21. Federoff, N. (1989) Maize transposable elements. In *Mobile DNA*. Berg, D.E. and Howe, M.M. (eds), American Society for Microbiology, p. 375.
22. Fiandt, M., Szybalski, W. and Malamy, M.H. (1972) Polar mutations in *lac*, *gal* and phage

λ consist of a few DNA sequences inserted eith either orientation. *Molec. Gen. Genet.* **119**, 223.
23. Fujiwara, T. and Mizuuchi, K. (1988) Retroviral DNA integration: Structure of an integration intermediate. *Cell* **54**, 497.
24. Gall, J. (1986) *The Molecular Biology of Ciliated Protozoa*. Academic Press, Orlando.
25. Glasgow, A.C., Hughes, K.T. and Simon, M.I. (1989) Bacterial DNA inversion systems. In *Mobile DNA,* Berg, D.E. and Howe, M.M. (eds), American Society for Microbiology, p.637.
26. Green, M.M. (1988) Mobile DNA elements and spontaneous gene mutation. In *Eukaryotic transposable elements as mutagenic agents*, *Banbury Report* **30**, 41.
27. Hayes, W. (1952) Recombination in *Bact. coli* K-12: unidirectional transfer of genetic material. *Nature* **169**, 118.
28. Hayes, W. (1953) Observations on a transmissible agent determining sexual differentiation in *Bact. coli. J. Gen. Microbiol.* **8**, 72.
29. Heffron, F. (1983) Tn*3* and its relatives. In *Mobile Genetic Elements*. Shapiro, J.A. (ed.) Academic Press, p. 223.
30. Hirsch, H.J., Saedler, H., and Starlinger, P. (1972a) Insertion mutations in the control region of the galactose operon of *E. coli*. II. Physical characterization of the mutations. *Molec. Gen. Genet.* **115**, 266.
31. Hirsch, H.J., Starlinger, P. and Brachet, P. (1972b) Two kinds of insertions in bacterial genes. *Molec. Gen. Genet.* **119**, 191.
32. Hu, S.,Ohtsubo, E., Davidson, N. and Saedler, H. (1975) Electron microscope heteroduplex studies of sequence relations among bacterial plasmids: Identification and mapping of the insertion sequences IS1 and IS2 in F and R plasmids. *J. Bacteriol.* **122**, 764.
33. Hutchison, C. A., Hardies, S.C., Loeb, D.D., Shehee, W.R. and Edgell, M.H. (1989) LINES and related retroposons: Long interspersed repeated sequences in the eucaryotic genome. In *Mobile DNA*. Berg, D.E. and Howe, M.M. (eds), American Society for Microbiology, p. 93.
34. Jacob, F., Brenner, S. and Cuzin, F. (1963) On the regulation of DNA replication in bacteria. *Cold Spr. Harb. Symp. Quant. Biol.* **28**, 329.
35. Jacob, F. and Wollman, E. (1961) *Genetics and the sexuality of bacteria*. Academic Press, New York.
36. Jordan, E., Saedler, H. and Starlinger, P. (1967) Strong polar mutations in the transferase gene of the galactose operon of *E. coli*. *Molec. Gen. Genet.* **100**, 296.
37. Jordan, E., Saedler, H. and Starlinger, P. (1968) O^o and strong polar mutations in the *gal* operon are insertions. *Molec. Gen. Genet.* **102**, 353.
38. Kleckner, N. (1983) Transposon Tn10. In *Mobile Genetic Elements*. Shapiro, J.A. (ed.) Academic Press, p. 261.
39. Kuhn, T. (1960) *The structure of scientific revolutions*. University of Chicago Press, Chicago.
40. Landy, A. (1990) Dynamic, structural and regulatory aspects of λ site-specific recombination. *Ann. Rev. Biochem.* **58**, 913.
41. Lederberg, J. and Tatum, E.L. (1946) Novel genotypes in mixed cultures of biochemical mutants of bacteria. *Cold Spr. Harb. Symp. Quant. Biol.* **11**, 113.
42. Malamy, M.H. (1967) Frameshift mutations in the lactose operon of *E. coli*. *Cold Spr. Harb. Symp. Quant. Biol.* **31**, 189.
43. Malamy, M.H., Fiandt, M. and Szybalski, W. (1972) Electron microscopy of polar insertions in the *lac* operon of *Escherichia coli*. *Molec. Gen. Genet.* **119**, 207.
44. McClintock, B. (1938) The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behavior of ring-shaped chromosomes. *Genetics* **21**, 315.

45. McClintock, B. (1950) The origin and behaviof of mutable loci in maize. *Proc. Nat. Acad. Sci. USA* **36**, 344.
46. McClintock, B. (1951) Chromosome organization and genic expression. *Cold spr. Harb. Symp. Quant. Biol.* **16**, 13.
47. McClintock, B. (1953) Induction of instability at selected loci in maize. *Genetics* **38**, 579.
48. McClintock, B. (1956a) Intranuclear systems controlling gene action and mutation. *Brookhaven Symp. Biol.* **8**, 58-74.
49. McClintock, B. (1956b) Controlling elements and the gene. *Cold spr. Harb. Symp. Quant. Biol.* **21**, 197.
50. McClintock, B. (1961) Some parallels between gene control systems in maize and in bacteria. *American Naturalist* **95**, 265.
51. McClintock, B. (1965) The control of gene action in maize. *Brookhaven Symp. in Biol.* **18**, 162.
52. McClintock, B. (1978a) Development of the maize endosperm as revealed by clones. In *The Clonal Basis of Development*, *Symp. Soc. Devel. Biol.* **36**, 217.
53. McClintock, B. (1978b) Mechanisms that rapidly reorganize the genome. *Stadler Genetics Symp.* **10**, 25.
54. McClintock, B. (1987) *The discovery and characterization of transposable elements: The collected papers of Barbara McClintock.* Garland, New York.
55. Mittler, J. and Lenski, R. E. (1990) Further experiments on excisions of Mu from *Escherichia coli* MCS2 cast doubt on directed mutation hypothesis. *Nature* **344:** 173-175.
56. Morse, M.L. (1954) Transduction of certain loci in *Escherichia coli* K-12. *Genetics* **39**, 984.
57. Morse, M.L., Lederberg, E.M. and Lederberg, J. (1956) Transductional heterogenotes in *Escherichia coli*. *Genetics* **41**, 758.
58. Shapiro, J.A. (1967) *The structure of the galactose operon in Escherichia coli K-12*. Doctoral dissertation, Cambridge University.
59. Shapiro, J.A. (1969) Mutations caused by the insertion of genetic material into the galactose operon of *Escherichia coli*. *J. Mol. Biol.* **40**, 93-105.
60. Shapiro, J. (1979) A molecular model for the transposition and replication of bacteriophage Mu and other transposable elements. *Proc. Nat. Acad.Sci. U.S.A.* **76**, 1933.
61. Shapiro, J.A. (1982) Mobile genetic elements and reorganization of prokaryotic genomes. In *Genetics of Industrial Microorganisms*. Ikeda, Y. and Beppu, T. (eds), Kodansha,Tokyo, p. 9.
62. Shapiro, J.A. (1983) *Mobile Genetic Elements*. Academic Press, New York.
63. Shapiro, J.A. (1984) Observations on the formation of clones containing *araB-lacZ* cistron fusions. *Molec. Gen. Genet.* **194**, 79.
64. Shapiro, J.A. (1985) Mechanisms of DNA reorganization in bacteria. *Int. Rev. Cytol.* **93**, 25.
65. Shapiro, J.A. (1991) Genomes as smart systems. *Genetica* **84**, 3.
66.Shapiro, J.A. (1992) Kernels and colonies: The challenge of pattern. In *The Dynamic Genome*, Federoff, N. and Botstein, D. (eds), Cold Spring Harbor Press, p. 213.
67. Shapiro, J.A. 1992. Natural genetic engineering in evolution. *Genetica*, in press.
68. Shapiro, J.A., MacHattie, L., Eron, L., Ihler, G., Ippen, K., Beckwith, J.R., Arditti, R., Reznikoff,W. and MacGillivray, R. (1969) The isolation of pure *lac* operon DNA. *Nature* **224**, 768.
69. Shapiro, J.A. and Leach, D. (1990) Action of a transposable element in coding sequence fusions. *Genetics* **126**, 293.
70. Sherratt, D. (1989) Tn3 and related transposable elements: Site-specific recombination and transposition. In *Mobile DNA*. Berg, D.E. and Howe, M.M. (eds) American Society for Microbiology. p. 163.

71. Swanson, J. and Koomey, J.M. (1989) Mechanisms for variation of pili and outer membrane protein II in *Neisseria gonorrhoeae*. In *Mobile DNA*. Berg, D.E. and Howe, M.M. (eds) American Society for Microbiology. p. 743.
72. Weiner, A.M., Deininger, P.L. and Efstratiadis, A. (1986) Nonviral retroposons: Genes, pseudogenes and transposable elements generated by the reverse flow of genetic information. *Ann. Rev. Biochem.* **55**, 631-661.
73. Taylor, A.L. (1963) Bacteriophage-induced mutation in *E. coli*. *Proc. Nat. Acad. Sci. USA* **50**, 1043.
74. Toussaint, A. and Resibois, A. (1983) Phage Mu: Transposition as a life-style. In *Mobile Genetic Elements*. J.A. Shapiro (ed), Academic Press, New York, p. 105.
75. Varmus, H. (1983) Retroviruses. In *Mobile Genetic Elements*. Shapiro, J.A. (ed), Academic Press, New York, p. 411
76. Varmus, H. and Brown, P. (1989) Retroviruses. In *Mobile DNA*. Berg. D. and Howe, M. (eds), American Society for Microbiology, Washington, D.C., p. 53.
77. Watanabe, T. (1963) Infectious heredity of multiple drug resistance in bacteria. *Bacteriol. Rev.* **27**, 87.
78. Wollman, E., Jacob, F. and Hayes, W. 1956. Conjugation and genetic recombination in *Escherichia coli*. *Cold Spr. Harb. Symp. Quant. Biol.* **21**, 141.
79. Woodruff, R.C. and Thompson, J.N. (1992) Have premeiotic clusters of mutation been overlooked in evolutionary theory? *J. evol. Biol.* **2**, 000-000.
80. Yao, M-C. 1989. Site-specific chromosome breakage and DNA deletion in ciliates. In *Mobile DNA*. Berg. D. and Howe, M. (eds), American Society for Microbiology, Washington, D.C., p.715.
81. Zinder, N.D. (1953) Infective heredity in bacteria. *Cold Spr. Harb. Symp. Quant. Biol.* **18**, 261.