The Uses of Chemistry in the Reorganization of Genetics

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As the review given in the Introduction makes clear, discussions of reduction in late twentieth century philosophy of science have had little to say about the role played by modes of representation in scientific discourse. Once we admit that modes of representation are conceptually essential, and not merely decorative, features of scientific thought and discourse, the question of what happens to modes of representation when scientific fields are brought into a novel alignment (involving various partial integrations and subordinations) becomes compelling. The case discussed in this chapter presupposes the "reduction" of biology to chemistry that produced molecular biology, and studies in particular the effect of translating problems that arose in genetics into the idiom of molecular biology, that is, a specific "reduction" of genetics to molecular biology. I try to show that this novel alignment of fields is accompanied by an important shift in modes of representation, and that the latter play a central role in the clarification and solution of the engendering problems.

Chemistry uses a broad spectrum of modes of representation, which includes the linear or one-dimensional conventions for chemical formulae in combination with natural language, as well as the graphic records of chromatography (various symbolic languages); and various kinds of picturing (iconic languages) from two-dimensional stereochemical formulae to three-dimensional models. It also makes use of other icons: those composed by light or electron microscopes, images produced by x-ray crystallography, and "imaginary" computer-generated images that propose the most likely disposition of the parts of a molecule. I would like to ask

what role these modes of representation play when a field like molecular biology borrows (and modifies) them, and then employs them in the service of other fields, in this case to solve a set of problems that genetics could formulate but could not solve on its own.

In the case study under consideration, the expository writings and experimental work of the molecular biologist Nina Fedoroff develop the work in genetics of Barbara McClintock, who discovered the phenomenon of the transposition of genes and thus threw into question the stability of the genome. Fedoroff "applied the new techniques of molecular biology to isolate and study McClintock's mobile maize genes," analyzing their structure and DNA sequences to deepen understanding of how these elements work, and to answer (and raise) new questions. In so doing, she at least temporarily puzzled and displeased McClintock. (Fedoroff, private correspondence) This was not so much because McClintock was committed as a geneticist to the study of complete organisms; the methods of the geneticist are after all highly abstract and reductive. And at that point in her life, while McClintock herself was not interested in trying to master a whole new set of techniques, she welcomed the application of molecular biology to her work. Rather, what made her uncomfortable with (for example) Fedoroff's inspired redescription of the significance of McClintock's work in the lead essay in *Mobile Genetic Elements*, edited by James Shapiro, was that it suggested substantive additions to, and in a sense corrections of, her work. (Fedoroff 1983a) In particular, McClintock wanted to view the relations among (to use Fedoroff's vocabulary) autonomous and nonautonomous transposing elements, like the *Ac-Ds* system, as a hierarchical regulatory system analogous to that of the lac operon. But this analogy was only superficial. Transposons do play some role in the regulatory systems that guide the development of the organism, but in a less central and more complex way than McClintock supposed. The vocabulary, instrumentation and lab techniques, and modes of

representation peculiar to molecular biology were needed to make precise the role played by transposons and the transposase they encode in the control of developmental parameters.

Nonetheless, despite McClintock's reservations, Fedoroff's recasting of her work did much to persuade the recalcitrant scientific community to take McClintock's ideas seriously. It was hard for most scientists to believe that a transposable segment of DNA could literally be inserted into another gene until the inserted nucleotide sequence was laid before their eyes -- Fedoroff was the first to demonstrate this for McClintock's elements (Fedoroff 1983b, 1983c, 1984) -- or until a biochemical mechanism, the activity of transposase in excising the transposon from its site, was suggested. That acceptance also depended on demonstrating that transposition was a widespread phenomenon, not just peculiar to maize. Indeed, the extension of McClintock's results to other organisms is the organizing principle of Shapiro's volume. But the phenotypical expression of transposition differs so much from organism to organism that it can be identified as the same kind of phenomenon only by appealing to evidence at the molecular level. In her (1999), Carla Keirns does an excellent job of explaining why the visual means employed by McClintock in her articles (mostly photographs of the maize kernels which were her primary evidence, as well as camera lucida images drawn from microscopic images of maize chromosomes in the pachytene stage) inhibited her ability to communicate with the broader community of scientists. In this essay, I will concentrate on why the broader spectrum of images employed by Fedoroff were more persuasive.

However, I would like to counter the suggestion that my study supports a reductionist understanding of the advance of science. As I see it, this episode in the history of science, where genetics and molecular biology intersect in complex ways, shows not that the study of genetics and the natural processes that drive evolution comes down to the sequencing of genomes, but

rather that the techniques of molecular biology have become an indispensable part of a repertory. To speak objectively, the organic world is organized in a stunningly multi-level fashion, worthy of the metaphysical vision of Leibniz, and every level has its characteristic unities, which in turn have their own characteristic effects. To paraphrase Rohrlich (1988, 304-5) and to speak more in terms of human epistemology, the characteristic parameters of the theories that study "coarser" objects cannot be retrieved from theories that study "finer-grained" objects. I will return to this point at the end of the essay; molecular biology supersedes but does not replace the field and greenhouse studies of genetics. (See L. Keller, 1999) And this seems to have been McClintock's view: discussing the latter's assessment of the operon explanation of gene regulation, Keirns argues, "McClintock's vision of life was in layers of complexity, so even if the operon were present in every organism, it did not begin to explain evolution, development, or organismal complexity. McClintock sought a new synthesis." (1999, 304)

2. Reduction

Philosophers of science who take their lead from logic rather than from the history of science have tended to talk about the relations between scientific domains as relations of logical derivation between formal theories. This assumes, of course, that a formal theory (a theory expressed in the language of first order logic, with the addition of vocabulary terms specific to the theory and axioms governing, or defining, those terms) could do justice to the activity of scientists in a scientific domain, and that once the terms characteristic of the "reduced" theory have been redefined in the vocabulary of the "reducing" theory the claims of the reduced theory could be derived as theorems from the axioms of the reducing theory. (See, for example, Hempel and Oppenheim 1948, Carnap 1937, Nagel 1961.) Moreover, the language of science is taken to

be both symbolic rather than iconic (there is no talk of pictures or picturing) and transparent, a neutral medium of description that adds nothing of its own to the conceptual situation. Finally, logicizing philosophers have tended to look at diachronic relations between predecessor and successor theories; the literature is studded with discussions of the logical relation between Newtonian Mechanics and Einstein's Theory of Relativity. (See, for example, Sklar 1967, Schaffner 1967, Nickles 1973.)

This account has come in for a great deal of criticism, on various grounds. Primarily, scientific domains are not merely theories; they include "a central problem, ...items taken to be facts related to that problem, general explanatory factors and goals providing expectations as to how the problem is to be solved, [experimental] techniques and methods, and sometimes but not always, concepts, laws and theories which are related to the problem and which attempt to realize the explanatory goals." (Darden and Maull 1977, 44) In general, domains (for example, genetics, biochemistry, and physical chemistry) do not compete, and one domain does not supplant another; rather, domains are brought into relation with each other by inter-domain theories and methods in the service of problem solving, and persist in relative autonomy. Also, of course, it makes no sense to talk about "deriving" the experimental techniques, items, or explanatory goals of one domain from those of another: only propositions may be derived from other propositions. To leave aside the importance of problems, problem-solving methods employed in the lab and the field, and the rich variety of modes of representation, is to make the rational reconstruction of scientific progress a hollow enterprise. (See Rohrlich 1988, Scerri 1991, Sarkar 1992, Grosholz and Hoffmann 2000.)

When a philosopher looks at the history of science as a series of problems to be solved (rather than as a set of completed theories to be set in inferential relation), certain features of the

advance of scientific knowledge leap to the eye. Scientific domains are often constituted around a paradigmatic problem, and just as often this problem proves to transcend the problem-solving abilities of that domain. Darden and Maull give a good example: "In genetics, the question arose: where are the genes located? But no means of solving that question within genetics were present since the field did not have the techniques or concepts for determining physical location; cytology did have such means." (1977, 50) Scientific domains become linked (but not reduced in the logicist sense just described) when one is called in to aid the other, against prior background knowledge that indicates there is an overlap in the phenomena they study. The items of linked domains may coincide, though each domain studies different aspects of them for different ends; or the items of one may serve as parts of the items of the other viewed as wholes, or as causes of the items of the other viewed as effects, or as underlying structures of the items of the other viewed as functions or processes or even behavior.

The work of Barbara McClintock in genetics, centered on her discovery of the surprising phenomenon of transposition, raised problems that McClintock herself could not solve, and could formulate only with difficulty or incompletely. A few such problems were: What is the chemical basis on which genes may be excised in transposition? And, what is the relation of processes of transposition to the processes of gene regulation (which leave the structure of the genome intact but turn genes off and on in transitory and reversible ways)? How precisely does the insertion of a transposon change the structure of the genome? The solution to and reformulation of these problems, which called for the importation of the theory, experimental techniques, and modes of representation of molecular biology and biochemistry, guided by nascent "inter-domain theories," finally persuaded the broader scientific community to see the central importance of the phenomenon of transposition.

3. McClintock's Studies of Maize

For the purposes of this essay, I want to pay close attention to McClintock's language, and the images in the articles that appeared when she first announced her discovery. McClintock used a technique that induced breakage in chromosomes, after which the broken arms fused with each other: these rearrangements disturbed the genome in significant ways, and brought about "an unusual and unexpected series of new mutants..., characterized by types of instability known in genetic literature as mutable genes, variegation, or mosaicism." (McClintock 1946, 178) The patterns on Indian corn are a striking example of such variegation; indeed, Indian corn is an especially apt experimental plant, for each ear, with its hundreds of kernels, is like the microbiologist's petri dish: each kernel represents the outcome of a distinct mating event, and the pigmentation leaves a highly "readable" record of genetic events in the development of the kernel's tissue. (Fedoroff 1983a, 2) In the 1930's, Marcus Rhoades had determined that a mutant gene can become unstable in the presence of another particular gene, that is, its instability is conditional upon the presence of another gene (Rhoades, 1938); but he never supposed the genes moved in the genome. Rhoades' predecessor, R. A. Emerson, had entertained the hypothesis that "distinct gene elements" might be transferred from one allele to another, but could not see how to pursue that hypothesis. (Emerson 1929)

In the mid-1940s, McClintock realized that the chromosome breakage she had been studying was occurring repeatedly at a single site, which she named the *Ds (Dissociation)* locus; she also realized that a second locus was needed for breakage at the *Ds* site to occur, which she named *Ac (Activator).* (McClintock 1946, 1947) Shortly thereafter, McClintock realized that *Ds* could move: "It is now known that the *Ds* locus may change its position in the chromosome...

One very clear case has been analyzed, and, through appropriate selection of crossover chromatids, strains having morphologically normal chromosome 9 have been obtained. As a consequence of this aberration, the *Ds* locus in these strains has been shifted from a position a few units to the right of *Wx* to a position between *I* and *Sh*. This is a very favorable position for showing the nature of the *Ds* mutation process." (1948, 158) She also surmises the transposition of the *Ac* locus on the basis of its interaction with the *Ds* locus: "The *Ac* locus may have been removed from its former position and inserted into a new position in chromosome 9 in a manner similar to that observed for the transposition of the *Ds* locus, described above. Because *Ac* induces breaks at specific loci and gives evidence of undergoing a specific breakage process itself, this latter explanation is not improbable." (1948, 159)

As Fedoroff summarizes, "Unstable mutations of the type analyzed by both Emerson and Rhoades could be understood as the result of transposable element insertions into a locus, from which it frequently transposed during development, restoring gene function. McClintock was able to make the connection between transposition of a genetic element, the *Ds* locus, and the origin of a mutable gene giving a variegated phenotype, because the particular *Ds* element she first isolated had a second property, chromosome breakage, by which she was able to track the *Ds* element independently." (Fedoroff 1998, 960) Soon thereafter, McClintock could show that *Ac* did indeed move. (McClintock 1949). These papers of the late 40s include no diagrams or images, only reports of the breeding experiments and the visible patterns on kernels that resulted from them, and comments about the accompanying cytological examination of the chromosomes. All the same, the import of her results, and the evidence on which her claims are based -- gleaned from experiments using perfectly conventional experimental methods -- are quite clear.

McClintock twice presented her results to the larger scientific community, once in the *Proceedings of the National Academy of Sciences* in 1950 and again in the widely read journal *Genetics* in 1953 (the year that Watson, Crick, and Franklin elucidated the double helical structure of DNA). The former is remarkable for its boldness and clarity. McClintock announces her thesis that transposition occurs, and that certain systems of autonomous and non-autonomous loci make possible a compelling explanation for "mutable loci" and the variegation or mosaicism they produce. "The origin and behavior of this mutable *c* locus has been interpreted as follows: Insertion of the chromatin composing *Ds* adjacent to the *C* locus is responsible for complete inhibition of the action of *C*. Removal of this foreign chromatin can occur. In many cases, the mechanism associated with this removal results in restoration of the former genic organization and action. The *Ds* material and its behavior are responsible for the origin and the expression of instability of the mutable *c* locus. The mutation-producing mechanisms involve only *Ds*. No gene mutations occur at the *C* locus; the restoration of its action is due to the removal of the inhibiting *Ds* chromatin." (1950, 350) She then forcefully suggests that since mutable loci have been recognized in a wide variety of organisms -- especially *Drosophila melanogaster,* that most crucial of all experimental beasts -- the occurrence of transposition must be considered not as peripheral or aberrant, but as central to the understanding of biological processes. "The author believes that the behavior of these new mutable loci in maize cannot be considered peculiar to this organism. The author believes that the mechanism underlying the phenomenon of variegation is basically the same in all organisms." (1950, 345)

The 1953 presentation in *Genetics* likewise includes no images, but instead a series of tables, six in all, that set out in perspicuous form her experimental crosses and the resultant

kernal phenotypes. (Figure 1 shows Tables 4 and 5.) The paper concludes, "Extra-genic units, carried in the chromosomes, are responsible for altering genic expression. When one such unit is incorporated at the locus of a gene, it may affect genic action. The altered action is detected as a mutation... The extra-genic units undergo transposition from one location to another in the chromosome complement. It is this mechanism that is responsible for the origin of instability at the locus of a known gene; insertion of an extragenic unit adjacent to it initiates the instability. The extragenic units represent systems in the nucleus that are responsible for controlling the action of genes. They have specificity in that the mode of control of genic action in any one case is a reflection of the particular system in operation at the locus of the gene." (1953, 598) She illustrates her claim by the example of the *Ds-Ac* system, and two loci it controls, *Sh (shrunken)* and *Wx (waxy)*. The control by the *Ds-Ac* system of the expression of the dominant and recessive forms of these traits is set out in her six tables. One thing to note in these tables is the ambiguity of the terms "*Sh"* and *"Wx"*" which stand for both a genic unit on the chromosome, and a phenotypical trait; by contrast, the terms "*Ds"* and *"Ac"* refer to a mobile extragenic unit on the one hand, and on the other -- at the phenotypical level -- no one trait, but rather a modality of the appearance of a trait. What "*Ds"* and *"Ac"* refer to phenotypically is rather abstract and elusive; indeed, "*Ds"* doesn't refer to anything on its own at the phenotypical level, since it is a non-autonomous element.

Logicizing philosophers of science who would like genetics to "reduce" to biochemistry or molecular biology must suppose that there is some kind of one to one map between terms of molecular biology (presumably referring to entities at the molecular level) and terms of genetics (presumably referring to entities at the visible, macroscopic level). But the present example reveals the oversimplifications involved in this view. First of all, the terms of molecular biology,

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like the terms of chemistry, refer ambiguously to both microscopic entities (macromolecules) and macroscopic entities (the "purified" materials that are sorted and analyzed in the molecular biologist's lab). So too do the terms of genetics, as we have just seen. Second, the theoretical move from structure to function (so characteristic of inter-domain theories and experimental practices) is typically much more complicated than any one-one mapping, for many structures may have no function or a diffuse function; some structures may have a function only in conjunction with other structures; some structures may be functional only in response to the environment that surrounds all the structures in question; and some of the same functions may be taken over by different structures.

Both these presentations, in two centrally important venues, seem to have been entirely ignored. As McClintock observed, rather dryly, "It was clear from responses to this report that the presented thesis, and evidence for it, could not be accepted by the majority of geneticists or by other biologists... I had already concluded that no amount of published evidence would be effective." (McClintock 1987, x; repr. in Fedoroff and Botstein, ed., 1992, 208) While it is quite probably true that the puzzled (and puzzling) reception to McClintock's ideas was due in part to the fact that she was a woman, and that she was attacking a central dogma of genetics, that is, the stability of the genome, these explanations do not go far enough. The acceptance and recognition that McClintock did in fact get all along (on a restricted scale, to be sure) was because she was recognized as a great scientist; no one ever denied she was a great scientist because she was a woman, though the fact that she was a woman probably led many scientists simply not to pay attention to her work. Moreover, we must wonder how the dogma about the stability of the genome got entrenched so quickly. (Shaw, private correspondence) Genetics as a scientific enterprise hardly existed before the work of Emerson and Rhoades, and their work

shows that examples of instability were known and studied from its inception. Why was there so little response to her work?

In 1965, J. D. Watson published a college textbook entitled *Molecular Biology of the Gene,* intended to report the state of the art to the educated public. The first chapter, entitled "The Mendelian View of the World," is an exposition of classical genetics, and ends with the observation, "In general, the tools of the Mendelian geneticists, organisms such as the corn plant, the mouse, and even the fruit fly, *Drosophila,* were not suitable for chemical investigations of gene-protein relations. For this type of analysis, work with much simpler microorganisms became indispensable." (1965, 31) This rather dogmatic statement or rather prophecy has proven unfounded; but it indicates that McClintock's organism, maize, was regarded as distinctly unfashionable. The second chapter is entitled "Cells Obey the Laws of Chemistry"; it presents the student with many chemical diagrams, including "small biological chemicals," like purine and pyrimidine, aromatic hydrocarbon, alcohol, nucleotide, amino acid, and sugar; "important functional groups," like phosphate, methyl, amino and carbonyl; and illustrations of oxidation and reduction, and various metabolic pathways. It ends with this programmatic claim: "Complete certainty now exists among essentially all biochemists that the other characteristics of living organisms (for example, selective permeability across cell membranes, muscle contraction, nerve conduction, and the hearing and memory processes) will all be completely understood in terms of the coordinative interactions of small and large molecules." (67) (One wonders if Watson intends "the hearing and memory processes" to include the works of Mozart and Proust.) The chapter summary ends with the modest claim, "So far the greatest impact on biological thought has come from the realization that DNA has a complementary double-helical structure. This

structure immediately suggested a mechanism for the replication of the gene, and initiated a revolution in the way biologists think of heredity." (69)

In the third chapter, "A Chemist's Look at the Bacterial Cell," the reader finds chemical diagrams of the twenty amino acids from which proteins are built, the four main nucleotide building blocks of DNA, and a portion of a polynucleotide chain. (Figure 2) Later on, the arrangement of genes on chromosomes is discussed, as well as the structure and function of DNA and RNA, the synthesis of proteins, and the replication of viruses. Despite an admiring discussion of the work of Jacob and Monod, operon theory and the theory of allosteric regulation in a chapter on protein synthesis and function, and despite an admission at the end of a chapter on cell differentiation that "virtually nothing is known about the molecular basis of the control of protein synthesis in the cells of the multicellular higher organisms," (438) Watson has absolutely nothing to say about McClintock's discovery of transposition. The terms "transposition" and "transposon" are not in the index of *Molecular Biology of the Gene,* and there are no citations of her work in the references given at the end of each chapter.

Watson's textbook shows the extent to which the study of genetics had been transformed by its complex new relations with physical chemistry, biochemistry, and molecular biology. It also suggests some reasons why Watson was unfamiliar with McClintock's work, ignored it, or found it unworthy of mention if he was aware of it. Watson's remarks about the relation of the study of living systems to chemistry, quoted above, reveal his commitment to a view of reduction which is totalizing and dogmatic: the geneticist must adopt the idiom of chemistry. McClintock, by contrast, was a geneticist, and thirty years older than Watson; while she was interested in biochemistry and molecular biology and a decade later welcomed its use in behalf of her theory of transposition, she herself was not about to pick up a new set of scientific tools.

Moreover, Watson may well have believed that McClintock's results were linked to an idiosyncratic feature of maize, an organism he regarded as peripheral in any case. If we recall that Watson was able to acknowledge the importance of the work of Jacob and Monod, we might note that what distinguished it from McClintock's is that it spoke in the idiom of molecular biology and was anchored in research on bacteria and viruses. Indeed, though McClintock had claimed explicitly that the phenomenon of transposition was to be found in many other organisms, the sameness underlying quite different phenotypic manifestations of transposition could only really be demonstrated by means of molecular biology.

4. Fedoroff's Translation of McClintock

McClintock needed a translator. One of the most faithful and successful was Nina Fedoroff, whose own work shows very clearly how such rewriting is not merely formal and certainly not trivial, for it substantively extends McClintock's results -- solving problems and allowing unforeseen problems to be formulated -- and in certain ways corrects them. In the late 1970s, Fedoroff was a postdoctoral fellow in the laboratory of Donald D. Brown, where the techniques of molecular biology were widely employed. The two papers (Fedoroff and Brown 1977, 1978) for example, map out the nucleotide sequence of an especially important gene cluster, "the repeating unit in the oocyte 5A ribosomal DNA" on the chromosomes of a frog, *Xenopus laevis*. The diagram that presents the final outcome of their work as a linear sequence of nucleotides includes a multiple articulation. (1977, 1196). (Figure 3) First, the sequence is distinguished into two regions, A and B, one of which is AT rich and one of which is GC rich. The first region is further divided into two regions which are quite stable, Al and A3, and a third region which may vary dramatically in length from instance to instance, A2. The second region

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is divided into three sections, region B1 (thought to encode directions that initiate and guide transcription); the gene itself; region B2 (which duplicates the end of A and B1); and the "pseudogene," which duplicates most of the gene but seems inactive.

Note how the display of the sequence, horizontal but also carefully given a vertical articulation, makes clear the duplications within it. In the (1978, 712) paper, the same strategy of using vertical as well as horizontal display to highlight duplications is employed. (Figure 4) Here, the strategy also displays possible deletions; that is, the mode of presentation makes visible not only what is present in the sequence but also what is -- arguably -- absent. The paper concludes that " 'spacers' like A, because they tend to enhance the overall duplication/deletion rate, may be critically important to both the stability and the evolutionary flexibility of the multigene family [the repeating unit A-B]." (713) Indeed, the interplay of duplication and deletion in the dynamic processes of evolution is the focal point of the discussion section at the end of (1978). The ability of discourse to represent negation, nothingness, absence, unrealized possibles, and so forth, is one of its most spectacular and troublesome features, as Plato was among the first to expound philosophically in the *Parmenides* and elsewhere. The chemist's Table of Elements, also a horizontal display with careful vertical articulation, illustrates the same point.

The linearity -- the rectilinearity -- of this sequence also suggests other philosophically interesting aspects of the representation here. What is odd about this representation is that it is iconic (it is a picture that looks like what it represents) but it appears to be symbolic. This is because what it pictures is itself symbolic: that is, the nucleotide sequence is a "linear" code that functions as a language that "refers to" things that it does not resemble. Both words I have just put in quotes deserve explanation. The way the nucleotide sequence "refers to" things is by

guiding the construction of messenger RNA, which in turn guides the construction of proteins with a complex spatial configuration. Thus the distinction between symbolic and iconic representations is blurred when the object represented (in this case the nucleotide sequence) has a communicative function that is carried out in a mediated fashion.

Yet while the nucleotide sequence CAAAGCTTCA... etc. is a picture, it is also a highly stylized picture that leaves out a great deal of the original chemical complexity, and also distorts it. And this distortion and stylization are due in part to our own human, Western, English conventions of the printed word. The lines in an English book are read from left to right, and a page of horizontal lines is read from top to bottom; so here, the direction in which ribosomes, for example, "read" parts of the nucleotide sequence, is depicted as moving from left to right, and also (line by line) from top to bottom. Moreover, the lines are straight. While the nucleotide sequence is "linear" in that its nucleotides are read one by one, in order, by the ribosomes that construct the messenger RNA, the "linearity" of CAAGCTTCA... is itself an artifact of certain conventions of writing. For the shape of DNA is certainly not a straight line. First of all, each nucleotide has its own complex chemical structure, which moreover sits in a complex chemical scaffolding (a repeating series of a phosphate group and a hydroxyl group); our representation elides all that for the sake of clarity. (See Figure 2) Second, the nucleotides-with-scaffolding sit in a double helix, which is itself wound around molecular bobbins and then braided and rebraided into chromatin, the material of which chromosomes are made. The straight lines of the printed nucleotide sequence are a tidy abstraction.

In the (1978) article, Fedoroff and Brown describe at length the experimental processes that allowed them to map the region A-B in *X. laevis* oocyte 5S DNA, citing in particular their adaptation of the methods of Sanger and Coulson (1975) and Maxam and Gilbert (1977). These

methods for determining nucleotide sequences are emphatically chemical, for they require

processes of purification, the cleaving of complex molecules by chemical means, the initiation

and termination of reactions, fractionation, electrophoresis, and autoradiography of the resultant

substances on acrilamide gel at the end. The description of the process, given in the Maxam and

Gilbert (1977) abstract summarizes: "DNA can be sequenced by a chemical procedure that

breaks a terminally labeled DNA molecule partially at each repetition of a base. We describe

reactions that cleave DNA preferentially at guanines, at adenines, at cytosines and thymines

equally, and at cytosines alone. When the products of these four reactions are resolved by size,

by electrophoresis on a polyacrilamide gel, the DNA sequence can be read from the pattern of

radioactive bands." (Figure 5) Fedoroff and Brown (1978) present a number of similar

autoradiographs, and describe in some detail how they were used to map out the A-B repeating

unit. (Figure 6) In a spin-off paper (1979), Fedoroff investigates the effects of the transposon

Tn9 (transposed from bacteriophage P1) when it is transposed into and deleted from the spacer

sequence (region A, above) which she and Brown had mapped out.

Towards the end of the postdoctoral phase of her research, Fedoroff met McClintock and

became deeply interested in her program, and decided to make use of her own skills in molecular

biology to take McClintock's research on maize "to the next stage." A series of studies of maize

followed, which include a description at the molecular level of the effects of the controlling

element *Ds* on the structure and expression of the *Sh* locus (Fedoroff, Mauvais, and Chaleff,

1983b); a report of the molecular similarity of *Ac* and *Ds* ("the 4. 1 kb *Ds* is almost completely

homologous to the Ac element, differing by a central deletion of less than 0.2 kb") in light of a

description of their insertion in the *Wx* locus (Fedoroff, Wessler, and Shure, 1983c); and a

determination of the full nucleotide sequence of *Ac* along with evidence that its "open reading

frame 1 " (the first of two) encodes a transposase, as well as evidence of strong structural similarity between the maize transposon and the bacterial transposon Tn3 (Fedoroff, Pohlman and Messing, 1984).

It also included an investigation of the maize *Spm* transposon which is involved both in processes of transposition and in a regulatory system, and thus provides an instructive case study of how these two distinct functions (transposition and regulation) may be interrelated. (Schlaeppi, Raina, and Fedoroff, 1994) The exposition of the Spm controlling element family plays an important role in the last pages of the essay "Controlling Elements in Maize" mentioned above (Fedoroff 1983a), that leads off the Shapiro volume (1983) designed to give a full dress presentation of McClintock's work on transposition to the scientific community. Investigating the *Spm* element at the molecular level, Fedoroff came to see that she must respectfully differ with McClintock, even as she tried to elaborate McClintock's program in genetics in terms of molecular biology.

The article begins with an extended discussion of the reproductive biology of maize, which counters Watson's claim that "organisms such as the corn plant, the mouse, and even the fruit fly, *Drosophila*, were not suitable for chemical investigations of gene-protein relations" by showing how and why the development of maize in fact allows it to wear genetically significant events on its sleeve, or more precisely, on its endosperm and pericarp (1983a, 1-11) It is full of pictures of corn kernels exhibiting various kinds of significant mosaicism. This is followed by an overview of the *Ac-Ds, Spm,* and *Dt* controlling element families, and then a long section on *Ds and Ac* (1983a, 16-40), where only a few images typical of molecular biology appear, although the footnotes lead from McClintock's own work to that of her successors, including Fedoroff.

The section on the *Spm* controlling element family (1983a, 40-53) and the concluding

pages counter or qualify a claim that McClintock often made in the 60s and 70s, namely that

systems of transposable elements were regulatory systems. For example, in a paper presented to

the Brookhaven Symposium on Genetic Control of Differentiation, "The Control of Gene Action

in Maize," McClintock writes, "That genetic mechanisms are involved in the control of actions

of genes is now well established," (162) and that in particular "This report will be concerned

mainly with control of gene action by regulatory systems whose elements have been identified

and characterized." And she concludes, "it should again be stated that limitations of space

preclude a comprehensive review of the properties of known controlling elements in maize, or of

the relation of the described systems to those in other organisms. It is hoped, nevertheless, that

the outlined modes of operation of these systems may serve to indicate not only their

extraordinary versatility in regulating gene action during development, but also their potential

economy. A number of different genes, related or unrelated in function, can come under the

control of a single regulatory system; and neither their times of action during development nor

the levels of their action at any one time need be the same." (1965, 181) Here McClintock

conflates the action of transposition and that of regulation, as if she hoped that transposition

would be the key to gene regulation.

But what Fedoroff (and others involved in similar research) discovered was that

transposition and regulation must be distinguished, and described at the molecular level, before

their interrelation or interaction could be properly discerned. At the end of "Controlling

Elements in Maize," Fedoroff writes, "McClintock focused on the interactions between

transposable elements and genes, attributing a regulatory function to the elements... Although it

is evident that controlling elements have genetic mechanisms for sensing developmental time

and position, the view that they are fundamental to gene regulation has not been widely accepted." (Fedoroff 1983a, 56) There are a few well-documented cases where the rearrangement of DNA is known to regulate gene expression- indeed, Fedoroff's own studies of *Spm* pointed in that direction. But Fedoroff emphasizes that this is a set of problems still to be explored, and not to be decided by fiat. Her (Schlaeppi, Raina, and Fedoroff, 1994), published a decade later, describes the regulatory function of the *Spm* transposon in molecular terms, a function which is clearly to be distinguished from its ability to transpose: "*Spm* is epigenetically inactivated by C-methylation near its transcription start site. We have investigated the interaction beween TnpA, an autoregulatory protein that can reactivate a silent *Spm,* and the promoter of the element. The promoter undergoes rapid de novo methylation and inactivation in stably transformed plants, but only if it includes a GC-rich sequence downstream of the promoter. TnpA activates the inactive, methylated promoter and leads to reduced methylation. By contrast, TnpA represses the active, unmethylated *Spm* promoter... TnpA is therefore a unique regulatory protein with a conventional transcriptional repressor activity and a novel ability to activate a methylated, inactive promoter." (Summary, 427). The figures in this article include both schematic representations of the nucleotide sequence and highly "chemical" charts of the results of an ongoing experimental research program. (Figure 7 includes Figures 2 and 3 from the article.)

5. Conclusion

In the early 1980's, Nina Fedoroff published two important expositions of Barbara McClintock's work, one (1983a, mentioned above) aimed at the scientific community, and another (1984) a popularization published in *Scientific American*. The spectrum of figures and illustrations that

Fedoroff employs in these articles reflect the bridging that she accomplished, in translating the work of McClintock into the idiom of molecular biology. For example, (1983a) begins with drawings of a maize kernel, mature plant, a pair of spikelets with anthers, and the growing egg cell in its embryo sac, as Fedoroff explains at length why maize was a particularly happy choice of experimental plant for McClintock. Figure 8 is a photograph of an ear of maize. In the pages that follow, by contrast, figures 4, 5, 6, 7, 9, and 10 are the "cartoons" of mechanisms that molecular biologists habitually use and which hover – like the formulae of chemists – between "summaries of experimental data and diagrams of real microstructure." (Keirns 1999, 177-80) The (1984) *Scientific American* article begins with many beautiful color illustrations of maize ears and individual kernels, and then turns to the "cartoons" of molecular biology, including especially impressive depictions of the *Ac* and several *Ds* elements of maize, and the mechanism of cycling *Spm*.

Fedoroff's molecular biology (as well as, of course, the work of other colleagues represented in [Shapiro 1983]) was able to answer certain questions that McClintock's genetics could formulate but not fully investigate. This is a pattern of a localized but not thoroughly global or completable relation between domains of research that Darden and Maull (1977) aptly dubbed "interfield theories". And so it is still true that genetics continues to generate problems (and solutions) that cannot be directly formulated or solved in terms of molecular biology, but must be addressed by other means. The "mechanisms" of selection appear to operate at the level of the genome (where there are biochemical processes analogous to reproduction), the individual, and the group. To investigate precisely the rates and effects of spontaneous mutation and its evolutionary consequences may, for example, require statistical methods (and a great deal of counting and measuring in the laboratory, field or greenhouse) to infer the genomic mutation

rate and distribution of mutational effects in successive generations of a large population of macroscopic individuals, taking into account differences in proliferation as well as differences in survival. (See, e.g., Shaw, Byers, and Darmo, 2000.) Much of this research may, and indeed must, be conducted without paying attention to changes at the molecular level, even when it deals with an organism whose genome has been completely "mapped."