DOI: 10.1534/genetics.110.120469

Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Mutagenesis as a Genetic Research Strategy

Raphael Falk[1]

Department of Genetics and Program for the History and Philosophy of Science, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel

ABSTRACT

Morgan's three students (Muller, Sturtevant, and Bridges) introduced reductionist empirical methods to the study of the chromosomal theory of heredity. Herman J. Muller concentrated on mutations, namely changes in the heterocatalytic properties of genes, without losing their autocatalytic (self-replication) properties. Experimental induction of mutations allowed quantitative analyses of genes' parameters, but hopes to deduce their chemicophysical character were never fulfilled. Once the model for DNA structure was proposed, the reductionist notions of mutation analysis were successfully applied to the molecular genes. However, it was soon realized that the concept of the particulate gene was inadequate. The more the molecular analysis of the genome advanced, the clearer it became that the entities of heredity must be conceived within systems' perspectives, for which special tools for handling large number of variables were developed. Analytic mutagenesis, however, continues to be a major strategy for the study of the cellular and chromosomal mechanisms that control mutation inductions.

This year, 2010, is the centenary of the publication of Thomas Hunt Morgan's article "Chromosomes and Heredity" in *The American Naturalist* (Morgan 1910) in which he introduced his chromosomal theory of inheritance. Five years later, in 1915, the book *The Mechanism of Mendelian Heredity* was published (Morgan *et al.* 1915). By that time Morgan's three students, Alfred H. Sturtevant, Herman J. Muller, and Calvin B. Bridges, had already taken the lead in developing the chromosomal theory of heredity, each applying a specific research strategy to their model organism *Drosophila melanogaster*. Bridges' strategy was to analyze segregation in genomic aberrations, such as X-chromosome nondisjunction, triploidy, and various aneuploidies; Sturtevant developed the concept of linkage as a tool for chromosome mapping (later extended by him also to developmental fate-mapping), whereas Muller's attention turned to the elucidation of the nature of the genes, the "ultramicroscopic particles," which according to him, were present in the *thousands* in the cell "besides the ordinary proteins, carbohydrates, lipoids, and extractives, of their several types" (Muller 1922).

Mutations are the mine of variability essential for the study of the hereditary components of any specific property. But mutations are also phenomena that provide information on the nature and function of the hereditary machinery *per se*. Here I wish to examine Muller's forceful conceptual and methodological impact in the decades prior to hands-on genomics on the utilization of mutations for elucidating the structural and functional properties of the entities of heredity and examine its unfolding in the later decades of the 20th century. I shall claim that the powerful reductionist conception of Muller, which provided experimental tools for early genetic analyses, also bestowed the conceptual framework for the establishment of molecular genetics. Eventually, however, it was from within molecular genetics that the inadequacy of Muller's conception was exposed: Modern genetic research overcame Muller's reductionist conception, though it still adheres—and will probably continue to adhere—to his strategy.

Reducing heredity to ultramicroscopic material entities: From early on, Muller's *conception* of the hereditary material was highly reductionist, more so than that of his two colleagues, and diametrically opposite to that of Morgan, who accepted reductionist *methods*—but not the conception—in his experimental analyses (see *e.g.*, Falk and Schwartz 1993; Falk 2009, part III; Green 2010). Muller explicated his conception of the gene and a working program for its elucidation in his important article of 1922 entitled "Variation due to

[1]*Address for correspondence:* Department of Genetics, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel.
E-mail: rfalk@cc.huji.ac.il

change in the individual gene" (Muller 1922): Heredity can be reduced to discrete material entities, genes, the properties of which may be logically deduced and thus examined experimentally. A distinctive property of the ultramicroscopic particles of heredity was self-propagation, a "very special series of physico-chemical effects upon its surroundings which produces . . . just this particular one, which is identical with its own complex structure."

> But the most remarkable feature of the situation . . . is the fact that, when the structure of the gene becomes changed, through some "chance variation," the catalytic property of the gene may become correspondingly changed, in such a way as to leave it still *auto*catalytic (Muller 1922).

This unique property of the predicted entities, that changes of their heterocatalytic function, actually functional errors, were not necessarily accompanied by loss of their autocatalytic properties—mutations—became Muller's handle for studying these entities, the genes. Mutagenesis became his research methodology for the rest of his life. True, from the beginning he hoped that one day "we may be able to grind genes in a mortar and cook them in a beaker after all," and that "we geneticists [would] become bacteriologists, physiological chemists and physicists, simultaneously with being zoologists and botanists." But initially and at the moment, it was the *genetic analysis of mutagenesis* that was best suited to elucidate the properties of genes, the atoms of heredity.

Defining the properties of the gene: Experimental mutagenesis was successfully introduced in 1927 by both Stadler (1928) and Muller (1927, 1929). However, Muller's added value achievement was in developing the *ClB* method for quantitative analysis of mutagenesis, thus allowing the empirical examination of the properties of the chromosomes and the deduced genes: The linear increase in the frequency of induced mutations with the dose of X rays indicated that genes were discrete entities in which single hit events were enough to induce "point mutations." For inducing aberrations, chromosome rearrangements like translocations, at least two breaks were needed, and indeed, higher power dose-effect hit-curves were observed. The frequency of induced rearrangements in spermatozoa was independent of the dose-rate of X rays, indicating that "healing" of broken ends could be studied independently of breakage; and normal chromosome ends—dubbed "telomeres" by Muller—had specific end properties that differentiated them from "sticky" broken chromosome ends. The discovery of Dipteran giant polytenic chromosomes in the early 1930s added another, cytological dimension to such mutagenetic analyses.

The climax of these analyses of mutagenesis was the monumental effort of Timoféeff-Ressovsky, Zimmer, and Delbrück in 1935 (Timoféeff-Ressovksy *et al.* 1935), of implementing the "target theory" to determine the physical parameters of the genes. The "target theory" was originally developed to estimate the dimensions of microscopic corpuscles like bacteria and enzymes from the dynamics of their inactivation by X rays. It appeared only natural to extend it to X-ray mutagenicity of discrete genes (Timoféeff-Ressovsky *et al.* 1935). However, arguably greater than the estimates of the dimensions of the genes was the impact of the work in turning the physicists' community's attention to biological issues. Delbrück, who wrote the theoretical section of the article, rephrased his conclusions in the language of quantum mechanics and perceived the gene in terms of "atomic associations" (*Atomverbände*) and electron states defined within contemporary physical theory.[2] This construction of the genes in terms of an "atomic physical model" induced Erwin Schrödinger to suggest in his booklet *What Is Life?* (Schrödinger 1944/1962) that genes were "aperiodic crystals" of chromosomal structures, in which mutations were "quantum jumps" of state of matter. Genes could now be discussed in terms of physics, and several physically trained scientists made genetics their business.

On the other hand, Muller's hope to extend experimentation to chemically induced mutations was disappointing. As put by Lotte Auerbach, one of the pioneers of chemical mutagenesis, the rationale of experiments with chemicals was: "If, as we assume, a mutation is a chemical process, the knowledge of the reagents capable of initiating this process should throw light . . . on the nature of the gene." But it soon turned out that the implicit reductionist assumption that chemical mutagens could directly affect genes as discrete entities, independently of the cell or, for this matter, the organism as a whole, could not be maintained. As we know, "the chemical nature of the gene has not been elucidated by research on mutation but in entirely different ways" (Auerbach 1967). Still, as appropriately termed by Elof A. Carlson, the target theory was "a successful failure" (Carlson 1966/1989): it emphasized the power of mutagenesis as an analytic tool and helped put the nature of the hereditary material at the focus of molecular biology.

Toward the molecular interpretation of heredity: To extend genetic research to bacteria and other prokaryotes that were better amenable to chemical and molecular experimentation, it was first necessary to show that these organisms obey the rules worked out for Drosophila, maize, and other eukaryotes. Luria and Delbrück's experimental analysis of bacterial mutations, which demonstrated that these were preadaptive (Luria and Delbrück 1943), together with Lederberg and Tatum's demonstration that bacterial genes may be

[2] I wish to thank Elof Carlson's personal communication: "When I asked Delbrück about the quantum model of mutation at a dinner at UCLA about 1965, he said "Ach, that was a silly piece of work!" It was a remark that I admired him for because he was as hard on himself as he was on the work of others."

arranged into linkage maps (Lederberg and Tatum 1946), indicated that Muller's and Sturtevant's strategies may be extended and also applied to bacteria. This provided evidence that their heredity is governed by the same system of discrete genes, linearly arranged in chromosomes, at which mutations occur independently of their function. Consequently, results of direct molecular analysis of bacteria (and their viruses) were relevant to the genetic theory that had been deduced in higher organisms.

Genetic analysis in microorganisms was for a while mainly an extension of that of higher organisms at greatly increased resolution power. And, as Jim Crow pointed out to me in a recent letter (April 26, 2010), "when the Watson-Crick model appeared, it shouted out the mechanism for the properties that Muller said a gene must have." Even after 1953, and the adoption of Watson and Crick's double helix model of DNA, there were relatively few experiments that directly involved the role of DNA molecules in genetics, such as that of Hershey and Chase's experiments of differential labeling of the progeny viruses with DNA's P^{32} atoms rather than with protein's S^{35} (Hershey and Chase 1952) or Meselson and Stahl's experiment of using heavy nitrogen to label newly synthesized polynucleotide chains (Meselson and Stahl 1958). Classic genetic analyses of mutagenesis remained a major tool for the examination of the organization of the discrete genes that were increasingly conceived in terms of entities of the DNA molecules. Seymour Benzer's extension of genetic analysis to the detailed mapping of *r*II mutants in T4 bacteriophages, unmistakably suggested the colinearity of the genetic and the molecular maps (Benzer 1955; Holmes 2000). Benzer and Freese's analysis of the mutagenicity of sites induced by nucleotide analogs 5-bromouracil (5BU) and 2-aminopurine (2AP) (and the pattern of their reverse mutations) allowed one to classify mutations into *transitions* (purine to purine or pyrimidine to pyrimidine mutations) and *transversions* (purine to pyrimidine or vice versa mutations) (Benzer and Freese 1958). Contrary to Auerbach and her colleagues' efforts, now the chemicals could be successfully applied (almost) directly to the target genes. This analysis was ingeniously extended by Brenner and colleagues to acridine-induced mutations that were conceived as additions or deletions of (single) nucleotides at the replication of the DNA molecule. The patterns of the induced mutations was interpreted in terms of a "general nature of the genetic code for proteins" (Crick *et al.* 1961) as frame-shift mutations. As already predicted by Gamow (1954) it was a code of triplets, three nucleotides per amino acid, and nucleotides are being read consecutively from given start points in open reading frames (ORF). This, together with the formulation by Crick of the "Sequence Hypothesis" and the "Central Dogma" of the unidirectional transfer of information from polynucleotide sequences to polypeptide sequences (Crick 1958), was—I wish to claim—the climax of the Mullerian reductionist conception of the gene as a discrete entity of heredity, and one of the climaxes of applying mutagenesis as an experimental analytic tool.

The end of the "golden age" of reductionist genetics: When shortly thereafter, Jacob and Monod introduced their model of regulation of β-galactosidase synthesis in *Escherichia coli*, although basically reductionist, it also contained—actually entailed—an anticlimax to the reductionist discrete gene conception, because mutagenetic analysis led them to suggest "operons" as higher-order genetic entities than the presumably discrete genes (Jacob and Monod 1961).

Although the golden age of reductionist microbial genetics of "what was true for *E. coli* was true for the elephant" was over, leading some to believe that this was the end of progress in the field (Stent 1969) and consequently turning them to other disciplines, mutagenesis was still a productive research strategy (see, *e.g.*, Falk 2009, part VII). However, from the 1970s onward, molecular genetic analyses became increasingly nucleotide-sequence oriented, starting with adding restriction fragment-length polymorphisms (RFLPs) to the array of genetic markers, and displaying nowadays whole genome sequences including their single-nucleotide polymorphisms (SNPs). Still, as Muller predicted, once genes were grinded in a mortar and cooked in a beaker, much of deductive genetic analysis became redundant. Geneticists went so far as to attempt a new kind of "bottom-up" reductionism, what they called "reverse genetics": Not anymore Mendelian-style *reverse deductions* from phenotype to genotype, rather analyses of DNA sequences and *direct inductions* from the properties of DNA sequences to phenotypes.

Accepting genes as autonomous entities of heredity extended the analytic power of mutagenesis as a research tool also in other directions. Most explicitly, it was employed by Beadle and Tatum in the formulation of their "one gene–one enzyme" doctrine for gene function and the consequent analysis of the genetic background of metabolic pathways in *Neurospora crassa* that they developed (Beadle and Tatum 1941a,b). Metabolic pathways in many organisms were broken down into discrete sequential steps by identifying mutations in genes that were each assigned to a specific enzyme.

Paradoxically, however, attempts to apply advanced molecular methods to induce the phenomena of heredity "bottom up" increasingly emphasized the "top-down" conception that discrete genes were nothing but empirically, scientists-demarcated splitting of the continuous nucleotide sequences of chromosome-long DNA molecules. Life has been increasingly conceived as a continuous phenomenon of complex interactive systems, inherently stabilized and constrained as integral wholes, whether that of the cell, of the organism, and even of the ecosystem. The insight of the complex interactive patterns of the genetic systems as integrated networks

rather than mega genomes, made the very conception of mutagenesis as an analytic *tool* for the identification of genes increasingly problematic. Yet, advanced techniques that were developed for handling simultaneously a great number of variables and integrate their functional organization, as well as analyzing quantitatively varying properties (QTLs) within the conception of systems analyses, allocated new meaning to the entities followed by mutagenesis. Top-down holistic "developmental systems approach" (DSA) conceptions of the genome have been increasingly adopted (see, *e.g.*, Neumann-Held and Rehmann-Sutter 2006).

There is no doubt that the increasing understanding of the role of genome sequences in the regulation of transcription of coding sequences, the processing of the transcripts and their translation, affected the relationship of geneticists to the traditional conceptions of embryology and development (see Britten and Davidson 1969), and the resurrection of Waddington's notion of epigenesis in development and eventually also in evolution (Jablonka and Lamb 1995). Top-down developmental constraints of organized systems became again part of biology as a unique system (see *e.g.*, Amundson 2005).

In the 1950s it was a major achievement of Jim Neel reducing the diverse patterns of the sickle-cell disease syndrome to pleiotropism of a single-gene effect of the erythrocytes (Neel and Schull 1954, pp. 170–172) and of Vernon Ingram locating the defect to the replacement of a single amino acid in the β-moiety of hemoglobin (Ingram 1963). It is only with the recent developments such as genome-wide association studies (GWASs) that the conceptual, almost "ideological" status of monogenic properties appears to have been irretrievably modified and phenotypic properties, whether human diseases or physiological properties of bacteria, are conceived as multidimensional complex interactive variables. Linus Pauling's concept of reducing genetic diseases to that of bottom-up "molecular diseases" (Pauling *et al.* 1949) was at the end replaced by the top-down notion of interacting genetic regulatory networks (GRN), which also interact with other components in the cell, thereby governing the rates at which genes are transcribed.

From mutagenicity as a research tool to mutagenicity as a research target: Although the use of mutagenesis as a tool for detecting genes that are involved in specific processes has not been abandoned, there are aspects where mutagenesis as the pivot of research strategy has not been exhausted, namely that of the property of mutagenicity itself. Quite early on, geneticists encountered lines with unusually high frequencies of mutations. Already in the 1920s Milislav Demerec genetically analyzed such a phenomenon in *Drosophila virilis* (and later, also in Delphinium). He demonstrated the involvement of several genes, which stimulate the mutability of the "miniature" gene in the flies' germ cells by >90% (Demerec 1926; Sinnott and Dunn 1932, pp. 176–177). Variation in mutation rates discovered in bacteria became a phenotypic property *per se*—"inherited mutagenicity"—the hereditary foundations of which grew to be the subject of genetic analysis, first in *E. coli* (see *e.g.*, Horst *et al.*1999) and then also in yeast and other eukaryotes. These studies obtained new quantitatively controlled dimensions with the discovery of repair mechanisms, primarily those of X-ray and ultra-violet–induced lesions (Witkin 1994).

As it turned out mutagenicity is a property of genes affecting recombination, breakage induction, and various mechanisms of breakage repair that are all involved in the fidelity of the household of DNA synthesis. Likewise, once it was realized that cancer is to a large extent a phenomenon of somatic mutations of the genome that are occupied in controls of cell division (and in their selection), it was natural that deep DNA-sequencing analyses would become the target of these effects (Bignell *et al.* 2010).

In conclusion: The great success of Morgan's chromosome theory of heredity was the application of reductionist research *strategies* to the study of its mechanisms. Through these, reductionist *conceptions* of a hereditary theory were established, which were upheld further by the achievements of analyses at the molecular level. The study of mutagenicity may be conceived as a happy-end meeting of Muller's efforts of mutagenesis as an indirect path of genetic analysis, and his dream "to grind genes in a mortar and cook them in a beaker after all." It was, however, from within molecular genetics that the inadequacy of the reductionist, bottom-up conception was exposed. Yet, analysis of mutagenicity is still a strategy that offers geneticists the tools for understanding the molecular controls of the fidelity of DNA at replication and upon repair.

I thank Bat-Sheva Kerem, Eran Meschorer, Giora Simchen, and especially Sam S. Schweber for their helpful discussions. An early version of this article was presented at the conference, *Mutagenesis: What It Means and How It Has Changed* at the Banbury Center, Cold Spring Harbor, NY, May 15–18, 2010.

LITERATURE CITED

Amundson, R., 2005 *The Changing Role of the Embryo in Evolutionary Thought: Roots of Evo-Devo*. Cambridge University Press, Cambridge, UK.

Auerbach, C., 1967 The chemical production of mutations. Science **158**(3895): 1141–1147.

Beadle, G. W., and E. L. Tatum, 1941a Experimental control of development and differentiation: genetic control of developmental reactions. Am. Nat. **75:** 107–116.

Beadle, G. W., and E. L. Tatum, 1941b Genetic control of biochemical reaction in *Neurospora*. Proc. Natl. Acad. Sci. USA **27:** 499–506.

Benzer, S., 1955 Fine structure of a genetic region in bacteriophage. Proc. Natl. Acad. Sci. USA **41**(6): 344–354.

Benzer, S., and E. Freese, 1958 Induction of specific mutations with 5-bromouracil. Proc. Natl. Acad. Sci. USA **44**(2): 112–119.

Bignell, G. R., C. D. Greenman, H. Davies, A. P. Butler, S. Edkins *et al.*, 2010 Signatures of mutation and selection in the cancer genome. (Report). Nature **463**(7283): 893–898.

Britten, R. J., and E. H. Davidson, 1969 Gene regulation for higher cells: a theory. Science **165:** 349–357.

Carlson, E. A., 1966/1989 *The Gene: A Critical History*. W. B. Saunders, Philadelphia.

Crick, F. H. C., 1958 On protein synthesis, pp. 138–163 in *Symp. Soc. Exp. Biol.,* Vol. 12. *The Biological Replication of Macromolecules*. Cambridge University Press, Cambridge, UK.

Crick, F. H. C., L. Barnett, S. Brenner and R. J. Watts-Tobin, 1961 General nature of the genetic code for proteins. Nature **192**(4809): 1227–1232.

Demerec, M., 1926 Mutable genes in *Drosophila virilis*. Proc. 4th Internat. Cong. Plant Sci. **1:** 943–946.

Falk, R., 2009 *Genetic Analysis: A History of Genetic Thinking*. Cambridge University Press, Cambridge, UK.

Falk, R., and S. Schwartz, 1993 Morgan's hypothesis of the genetic control of development. Genetics **134**(3): 671–674.

Gamow, G., 1954 Possible relation between deoxyribonucleic acid and protein structure. Nature **173:** 318.

Green, M. M., 2010 2010: a century of Drosophila genetics through the prism of the white gene. Genetics **184**(1): 3–7.

Hershey, A. D., and M. Chase, 1952 Independent functions of viral protein and nucleic acid in growth of bacteriophage. J. Gen. Phys. **36**(1): 39–56.

Holmes, F. L., 2000 Seymour Benzer and the definition of the gene, pp. 115–155 in *The Concept of the Gene in Development and Evolution*, edited by P. J. Beurton, R. Falk and H.-J. Rheinberger. Cambridge University Press, Cambridge, UK.

Horst, J.-P., T.-H. Wu and M. G. Marinus, 1999 *Escherichia coli* mutator genes. Trends Microbiol. **7:** 29–36.

Ingram, V., 1963 *The Hemoglobin in Genetics and Evolution*. Columbia University Press, New York.

Jablonka, E., and M. J. Lamb, 1995 *Epigenetic Inheritance and Evolution: The Lamarckian Dimension*. Oxford University Press, Oxford, UK.

Jacob, F., and J. Monod, 1961 Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. **3:** 318–356.

Lederberg, J., and E. L. Tatum, 1946 Gene recombination in *Escherichia coli*. Nature **158:** 558.

Luria, S. E., and M. Delbrück, 1943 Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28:** 491–511.

Meselson, M., and F. W. Stahl, 1958 The replication of DNA in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **44:** 671–682.

Morgan, T. H., 1910 Chromosomes and heredity. Am. Nat. **44:** 449–498.

Morgan, T. H., A. H. Sturtevant, H. J. Muller and C. B. Bridges, 1915 *The Mechanism of Mendelian Heredity*. Henry Holt, New York.

Muller, H. J., 1922 Variation due to change in the individual gene. Am. Nat. **56:** 32–50.

Muller, H. J., 1927 Artificial transmutation of the gene. Science **66:** 84–87.

Muller, H. J., 1929 The gene as the basis of life. Proceedings of the International Congress of Plant Sciences, Ithaca, NY. Vol. 1: 897–921.

Neel, J. V., and W. J. Schull, 1954 *Human Heredity*. University of Chicago Press, Chicago.

Neumann-Held, E. M., and C. Rehmann-Sutter (Editors), 2006 *Genes in Development: Re-Reading the Molecular Paradigm*. Duke University Press, Durham, NC.

Pauling, L., H. A. Itano, S. J. Singer and I. C. Wells, 1949 Sickle cell anemia, a molecular disease. Science **110:** 543–548.

Schrödinger, E., 1944/1962 *What Is Life? The Physical Aspect of the Living Cell*. Cambridge University Press, Cambridge, UK.

Sinnott, E. W., and L. C. Dunn, 1932 *Principles of Genetics*. McGraw-Hill, New York.

Stadler, L. J., 1928 Mutations in barley induced by X-ray and radium. Science **68:** 186–187.

Stent, G. S., 1969 *The Coming of the Golden Age: A View of the End of Progress*. Natural History Press, New York.

Timoféeff-Ressovsky, N. W., E. G. Zimmer and M. Delbrück, 1935 Über die Natur der Genmutation und der Genstruktur. (About the nature of gene mutation and gene structure.) Nachricht. Biolog. Gesell. Wissen., Göttingen **1:** 189–245.

Witkin, E. M., 1994 Roots: mutation frequency decline revisited. BioEssays **16:** 437–444.