Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

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The Gene (H. J. MULLER 1947)

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"THE Gene" was H. J. MULLER's Pilgrim Trust lecture, delivered before the Royal Society of London on November 1, 1945. World War II was barely over, but sea travel was still hazardous. A storm had dislodged a number of floating mines, and the transit to port of *SS Queen Mary* was something of an adventure (CARLSON 1981). Published in 1947, "The Gene" is the finest exposition of the state of development of genetics at the very dawn of its turning molecular in the decade spanned by AVERY, MACLEOD and MCCARTY (1944) and WATSON and CRICK (1953). The explosive transition was of course closely linked to contemporaneous history: the burgeoning commitment to scientific research, now strongly supported by government in a style inherited from wartime experience. I will be reviewing this paper in a retrospective mood: "now" (as opposed to "today") will mean 1945. The contrast of 1945 with 1991 gives us a chance to reflect how much we have learned in 46 years, and how much was anticipated. It is especially instructive to reread this work in company with DUBOS's *The Bacterial Cell* (1945), a noted microbiologist's synthesis that almost converges with "The Gene."

MULLER's leading argument is whether there is "even such a thing as genetic material at all, as distinct from other constituents of living matter." He responds that the simplest observation of the developmental life cycle points to some conserved invariant that persists from fertilization, through embryonic development and the formation of gametes, returning to the fertilized egg. This is then complicated by the requirement for accurate duplication of that invariant, whatever it may be, under its own influence. Discrete mutations are then further evidence of correspondingly discrete particles as the material basis of inheritance. However, the knowledge of chromosomes now enables an appeal to much more direct pragmatic evidence, if not yet of the material composition of the gene, at least of its cytological location. Most of the genetic research during 1900–1945 was indeed devoted to chromosome mechanics; today we view Mendelian ratios less as a fundamental law of biology than of the idiosyncrasies of chromosome partition in material carefully chosen for the avoidance of particularities like meiotic drive, nondisjunction, gene conversion or paternal imprinting.

MULLER turns to the chemical composition of chromosomes as predominantly "nucleoprotein, a compound of protein with nucleic acid, [as] was shown in analyses of sperm chromosomes by MIESCHER, 1897." The reference is to a compilation of MIESCHER's work for the previous 30 years. MULLER remarks that "only recently has it become reasonably certain–through the analogous finding in viruses–that it is really this major component rather than some elusive accompaniment of it which constitutes the genetic material itself." Protein, rather than monotonous nucleic acid, is presumably the information-bearer; however, "nucleic acid also exists in highly polymerized form . . . as may be very significant."

Much of MULLER's own research had concerned mutagenesis, including that induced by X-rays. Again, the gene is a particle with highly circumscribed locality. Mutation can alter one allele and leave its homologous partner "lying but a fraction of a micron away . . . undisturbed." "Blindness and molar indeterminacy" characterize mutation. How this can lead to constructive evolution is usually through the action of natural selection on ensembles of mutations each with small effect, and therefore unlikely to be disastrous. MULLER reaches hard to extract useful hints on the chemistry of the gene from X-ray mutagenesis. At least it is internally nonrepetitive or "aperiodic" (per SCHRÖDINGER; but most molecules are), on the feeble argument that mutation is a discrete event, not a protracted instability that might speak for continued internal reshuffling.

News of chemical mutagenesis was just trickling in, especially of the war gas, mustard (AUERBACH, ROBSON and CARR 1947). These studies were inspired by similarities between mustard gas burns and radiation damage. MULLER was aware of the mustard gas work at the time of his lecture, but military restrictions prevented his mentioning it (CROW 1990). MULLER also recited more dubious claims from Caltech of antibody-induced mutations in Neurospora (EMERSON 1944) which, together with PAULING and CAMPBELL's (1942) claims for antibody synthesis by protein folding *in vitro*, have been consigned to oblivion. Alkylation mutagenesis remains a lively research topic today and, indeed, "These . . . experiments constitute the first decided break in the impasse that had developed in studies directed toward the chemistry of the mutation process." Nevertheless, genetic chemistry has contributed more to the rather intricate and still problematical mechanism of mutagenesis than the converse (DRAKE 1989).

Historically, the validation of chemical mutagenesis seems long overdue, considering that almost every molecule is suspect today. This can be attributed in part to the tediousness of methods, requiring elaborate statistical validation, before bacterial systems were developed. Furthermore, few of MULLER's contemporaries were intellectually positioned to be able to marry concepts from genetics and chemistry; MULLER was by no means a sophisticated chemist, but used an aggressive and insightful imagination in borrowing from the insights of other disciplines.

The new horizon of chemical mutagenesis offered no obstacle as yet to the concept of evolutionary indeterminacy. Despite effects on "the frequency of gene mutation in general, . . . each individual mutation remains a chance and uncontrollable event, from the macroscopic standpoint." This has remained genetic orthodoxy to the present day, bolstered by revulsion about the criminal excesses of the Lysenkoist counter-doctrine. It deserves reexamination in the light of the intricacies of DNA conformation and its secondary structure, which are indisputably coupled to regulated gene expression (DAVIS 1989; LEDERBERG 1989). MULLER's consideration of heterochromatin position effect as a *cis*-acting influence of chromatin coiling on gene expression is a harbinger of today's second look.

Despite the molar indeterminacy of evolution, and the disruptive "bad" consequence of most mutations, "the Maxwell demon of natural selection. . . brings order out of mutation's chaos despite itself." MULLER could not yet know of the plethora of phenotypically silent mutations in DNA which today support a much greater role of mutation pressure and genetic drift in evolution (KIMURA 1991).

MULLER then turns to nonchromosomal genes. Chloroplasts are the best worked out; but animal cells can do without them. Uniparental inheritance constrains the diversification of chloroplast genomes, and their limited content suggests they have a correspondingly small role in evolution. The chloroplast probably "had a common ancestry with the chromosomal genes, dating back to the period before the latter had become organized into typical nuclear chromosomes." This conjecture was voiced on the brink of a new and successful cycle of evolutionary attribution of chloroplasts to endosymbiotic cyanobacteria (LEDERBERG 1952; MARGULIS 1981). LINDEGREN and SPIEGELMAN's yeast "cytogenes" are also mentioned, but with cautious reservations about the "links of the evidence" for the regular production of self-reproducing replicas from a chromosomal gene. This caution was amply vindicated. However, proviruses, from lambda to HIV today, securely occupy that niche. Some exceptional instances of gene amplification may follow a similar pattern (STARK *et al.* 1989).

For nonchromosomal genes in animal cells, MULLER attends to DARLINGTON and ALTENBURG's speculations about plasmagenes and viroids. These had some support from SONNEBORN's work on kappa in Paramecium, and this is extensively discussed. But while "The Gene" was in press, SONNEBORN revised his prior formulation of a chromosomal origin of kappa, and MULLER footnotes this. The polemics about such particles being viruses, symbionts or genes were the immediate stimulant for the overarching concept of plasmids (LEDERBERG 1952), which has largely dissolved the controversy.

Gene duplication within the chromosome is uncontroversial. After subsequent divergent mutation, "the germ plasm becomes not merely more compound but more complex and . . . the possibilities of organizational complexity for the body in general should rise also."

MULLER was the first high peer in genetics to enunciate that "virus particles . . . which fulfil the definition of genes in being self-determining in their reproduction and capable of transmitting their mutations, are composed of . . . nothing but nucleoprotein." He finds appealing DELBRÜCK's early ideas (1941) about polypeptide template-directed assembly "by means of a resonance . . . at peptide links . . . followed up by a finishing up of the peptide connections, and associated undoing of the" bonding of the old and new chains. To be sure, there is no evidence that gene synthesis involves peptide links; and protamines are too simple. However, some kind of steric complementarity might pertain between basic proteins and acid DNA. By 1953, WATSON and CRICK would model DNA-DNA complementarity as the core of modern molecular genetics. In 1947, chromosome synapsis was a possible clue to recognitional mechanisms in gene duplication,

though MULLER is quick to emphasize that karyological synapsis involves forces over microscopically visible distances. Especially perplexing (then and today) is meiosis in Neurospora, where "the chromosomes come into contact while still in a condensed, closely coiled condition . . ." MULLER's response here is an invocation of temporal vibrations, a premonition of holography, better left without further comment, though similar ideas still recur in neurobiological speculation. Many aspects of synapsis, and the example of Neurospora's ability to scan for duplicated segments (SELKER 1990), remain a challenging mystery today.

Akin to virus replication is pneumococcal transformation. MULLER's endorsement was an important testimonial for geneticists of that decade:

> In my opinion, the most probable interpretation of these virus and Pneumococcus results then becomes that of actual entrance of the foreign genetic material already there, by a process essentially of the type of crossing over, though on a more minute scale . . . that is, there were, in effect, still viable bacterial "chromosomes" or parts of chromosomes floating free in the medium used. These might, in my opinion, have penetrated the capsuleless bacteria and in part at least taken root there, perhaps after having undergone a kind of crossing over with the chromosomes of the host. In view of the transfer of only a part of the genetic material at a time, at least in the viruses, a method appears to be provided whereby the gene constitution of these forms can be analyzed, much as in the cross-breeding test on higher organisms. However, unlike what has so far been possible in higher organisms, viable chromosome threads could also be obtained from these lower forms for *in vitro* observation, chemical analysis, and determination of the genetic effects of treatment.

This emboldened me to posit a close analogy to the newly discovered phenomenon of genetic exchange in bacteria (LEDERBERG 1947). But he could not yet accept that the transforming activity had been proven to be pure DNA (contra "nucleoprotein"). PHOEBUS LEVENE had laid the groundwork of DNA chemical structure with the elucidation of the constituent deoxyribonucleotides and their linkage through phosphotriester bonds. But the model closest to hand was that of a monotonous tetranucleotide, which left little room for genetic informational variety. MULLER left several hints that larger polymers might alter our perceptions, but he had no platform for more detailed chemical modeling or experiment.

How do genes work? MULLER cautions against too facile a depiction of the gene or its primary product as an enzyme. I translate his two arguments: that the known primary gene product is another gene, and this has properties not shared by known enzymes; and that developmental pathways will almost always show pleiotropic complications, *viz.* several genes affecting one enzyme even if this is not seen in initial surveys ("new methods will be needed before the primary gene products can be identified"). I shared this skepticism about the ultimate rigor of the "one-gene:one-enzyme" theory, to the irritation of BEADLE and HOROWITZ (LEDERBERG 1956). In retrospect, it was an indispensable heuristic, and complications like the intervention of mRNA, RNA splicing and editing, and post-translational modifications could be left for later historical superimposition on the initial skeleton of colinearity of DNA with protein.

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ABOLINS L	EXP CELL RE		3	1	52
ANDERSON TF	BOTAN REV		15	464	49
BEADLE GW	ANN R BIOCH	R	17	727	48
"	ANN R PHYSL	R	10	17	48
"	FORTSCH CH		5	300	48
BLUMEL J	P NAS US		34	561	48
BROWN GB	ANN R BIOCH	R	22	141	53
BURT NS	BLOOD		6	906	51
CHAYEN J	NATURE		171	472	53
COULSON CA	T FARAD SOC		48	777	52
DAVIDSON JN	ANN R BIOCH	R	18	155	49
DEVI P	NATURE	L	160	503	47
ESSRIG IM	N ENG J MED	R	240	15	49
FRANKEL OH	HEREDITY		4	103	50
GULLAND JM	COLD S HARB		12	95	47
HARRISON JA	ANN R MICRO		1	19	47
HERSHEY AD	ADV GENETIC		5	89	53
HOFFMANN.H	Z NATURFO B		6	63	51
HOROWITZ NH	ADV GENETIC	R	3	33	50
HUSKINS CL	AM NATURAL		81	401	47
"	NATURE		161	80	48
JEHLE H	P NAS US		36	238	50
"	SCIENCE	M	111	454	50
KNIGHT CA	ADV VIRUS R	R	2	153	54
LANHAM UN	AM NATURAL		86	213	52
LAVELLE A	ANAT REC		119	305	54
LEDERBER.J	COLD S HARB		16	413	51
"	GENETICS		32	505	47
"	PHYSIOL REV	R	32	403	52
LEWIS EB	ADV GENETIC		3	73	50
LILLIE RS	AM NATURAL		82	5	48
LWOFF A	ANN IN PAST		78	711	50
MAZIA D	P NAS US		40	521	54
MEDAWAR PB	BIOL REV	R	22	360	47
MULLER HJ	AM J HU GEN		2	111	50
PONTECOR.G	ADV ENZYMOL		13	121	52
"	ADV GENETIC		5	141	53
"	SYM SOC EXP		6	218	52
ROPER JA	NATURE	L	166	956	50
SCHMITT FO	ANN R PHYSL	R	10	1	48
SPARROW AH	AM J BOTANY		34	439	47
SPIEGELM.S	COLD S HARB		12	211	47
"	SYM SOC EXP		2	286	48
STONE WS	P NAS US		33	59	47
VOGT M	Z INDUKT AB		83	324	50
WATSON JD	COLD S HARB		18	123	53
WITKIN EM	"		12	256	47
ZINDER ND	J BACT		64	679	52

FIGURE 1.—*Science Citation Index* to MULLER (1947) for the years 1947–1954 (reprinted from the *Science Citation Index® 1945–54 Cumulation* with permission of the Institute for Scientific Information® (ISI®), ©Copyright 1991). The most recent recorded citation is in ROBERT H. HAYNES' (1989) presidential address to the International Congress of Genetics.

MULLER was among the first to extrapolate from basic scientific knowledge of genetic mutation and evolution to their human implications. Mutational disorder will eventually afflict the human genome as a result of the blunting of natural selection by culture; but this process will take centuries and we have time to educate ourselves in countermeasures of genetic hygiene. His estimate of one lethal equivalent as the

genetic load of recessive mutation in the contemporary human still stands.

Meanwhile, we should be cautious about exposure to X-rays and to "special chemicals." It is curious that he makes no reference here to nuclear explosions. Fallout was yet to enter the lexicon (after the H-bomb tests), and even then MULLER was concerned that its effects might be exaggerated in contrast to other radiation hazards, and in ways that might erode nuclear deterrence of Soviet aggression (CARLSON 1981).

Horrified by the "terrible Nazi perversion of genetics," he believes that "any conscious guidance over our own genetic processes" be deferred for voluntary concern, understanding, and better developed social consciousness. Many psychological traits, in particular, are attributed to "training or by largely unwitting conditioning." But eventually social wisdom should allow "the self-reproduction of the gene and the self-reproduction of intelligence [to] reinforce one another in an ascending curve."

It will illustrate the impact of this article to list the citations that appeared in 1947–1954 (Figure 1).

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