Perspectives

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Ultraviolet-Induced Mutation and the Chemical Nature of the Gene

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TEXTBOOKS tell us that it was generally believed that genes were made of protein until the demonstration by AVERY, MACLEOD and MCCARTY (1944) that the material that caused bacterial transformation was DNA. Most textbooks fail to note that studies done several years earlier on mutations induced by ultraviolet (UV) radiation also indicated that DNA had genetic activity. And it is often forgotten that neither the UV experiments nor the identification of the transforming principle really convinced scientists that genes were made of DNA.

The first convincing demonstration of the mutagenic action of UV was made in *Drosophila* by EDGAR ALTENBURG (1934). His close colleague, H. J. MULLER, had shown that X-rays induced mutations in *Drosophila*, but that natural sources of ionizing radiation could account for only a small fraction of the spontaneous mutations (MULLER and MOTT-SMITH 1930). Therefore, ALTENBURG set about testing other kinds of natural radiation for a possible role. UV seemed to him a good candidate.

ALTENBURG first attempted to monitor UV-induced mutation by treating mature males and scoring sex-linked recessive lethals by MULLER's CLB procedure. He immediately ran into trouble administering the UV to the testes, because so much of the radiation was absorbed by the superficial tissues. (Perhaps this should have forewarned him that UV could not be responsible for much of the spontaneous *germinal* mutation in animals.) ALTENBURG (1934) adopted a procedure developed by GEIGY (1931) for irradiation of *Drosophila* eggs at the polar cap end; these pole cells are destined to give rise to the germ cells. With this material, ALTENBURG was able to show highly significant frequencies of recessive lethal mutations resulting from UV treatment. However, most of the further work on UV-induced mutations was done, not on *Drosophila*, but on other forms whose naked germ cells were readily accessible: pollen cells, fungus spores, and bacteria.

During the late 1930s, three separate investigators set up experiments to determine the *action spectrum* of UV-

induced mutation. The experiments involved measuring the mutagenic action of monochromatic UV, comparing the effect of the same dose for many different wave lengths. The results could be collected on a graph, plotting wave length *vs.* mutagenicity (see Figure 1). This action spectrum of mutation could then be compared with the UV absorption spectra of substances known to be present in chromosomes (proteins and nucleic acids). The logical assumption was that the substance that produced mutations when it absorbed UV radiation must be the genic material.

Experiments of this type were reported by L. J. STADLER (1939) on mutations induced in maize pollen, by HOLLAENDER (1939) on mutations in spores of the fungus *Trichophyton*, and by KNAPP and SCHREIBER (1939) on the spermatozooids of the liverwort *Sphaerocarpaceus*. All three studies concluded that the most effective wave lengths for the induction of mutations were those with maximum absorption by nucleic acid (and not by protein). It is not known whether any of them was aware of the work of the others before all of them reported their results at the International Congress of Genetics in Edinburgh in 1939, nor whether any of them had known beforehand of an earlier preliminary experiment of the same kind on *Antirrhinum* pollen by NOETHLING and STUBBE (1936).

The maize experiments have been described in a memoir by ROMAN (1988). This material had the advantage of an extensive set of known genes for endosperm traits that could be scored independently for mutations. This permitted STADLER to note a high frequency of coincident mutations (in the same pollen cell), which meant that some nuclei were receiving a larger dose than others. This alerted him to the unwanted complication that the nuclei were asymmetrically located in the pollen cells and the superficial layers were absorbing much of the incident UV. This required a rather complex correction to convert incident UV to actual dose.

The other two materials were not endowed with known mutable loci, but had the advantage of avoiding the complications of absorption by superficial tissues. The *Sphaerocarpaceus* spermatozooids provided especially

FIGURE 1.—Results of studies on the action spectrum of UV mutation as presented by LEA (1947). The dashed lines show the observed mutation rates in corn pollen (A), fungus spores (B), and liverwort spermatozooids (C). The solid curves show the absorption spectrum of DNA.

good material in this regard, being almost completely naked nuclei (KNAPP and SCHREIBER 1939). The investigators verified that the observed changes were gene mutations by their 2:2 segregations in tetrads.

LEWIS STADLER (left) in the cornfield with HERSCHEL ROMAN, about 1940.

ALEXANDER HOLLAENDER (left) at a Gatlinburg Symposium with the Wisconsin photochemist FARRINGTON DANIELS, 1952. Photo from the University of Wisconsin-Madison Archives.

The mutations scored in the experiments with fungus spores were changes in pigmentation or morphology of the colonies. These studies are discussed in an obituary of HOLLAENDER by SETLOW (1987), who notes that the authors showed extreme caution in their conclusions: "It is probably somewhat dangerous to overemphasize the importance of nucleic acid in the study of radiation effects on living cells. It is very well possible that in radiation produced mutations, the nucleic acid is only the 'absorbant' agent, then transfers the absorbed energy to the protein closely associated with it" (HOLLAENDER and EMMONS 1941). [For more information on HOLLAENDER's career, see the recent memoir by VON BORSTEL and STEINBERG (1996).]

The authors of the other studies were no more bold in their conclusions. STADLER (1939) at first worried that the apparent fit to nucleic acid absorption might be an artifact caused by the superficial absorption: "Unfortunately, the material is not suited to a rigorous physical analysis, and it is to be hoped that similar determinations of genetic effect may be made in material in which the dosage at the chromosomes may be more accurately determined." Later (STADLER and UBER 1942), after further refinements of the analysis still gave the same result, he was slightly more bold: "Considering the various approximations involved, the agreement (to the absorption spectrum of DNA) is surprisingly good and lends further support to the frequently advanced hypothesis that nucleic acid is intimately associated with the functioning of the germinal material."

KNAPP and SCHREIBER (1939), whose experiments with *Sphaerocarpus* clearly showed a nucleic acid spectrum for mutation induction, concluded "that the absorption of thymonucleic acid has a significant meaning in the mutagenic effect of UV." But they were quick to add, "It does not seem proven that thymonucleic acid is part of the organization of the gene."

JUDSON (1993), in a short, insightful paper that was

part of a symposium observing the 40th anniversary of the WATSON-CRICK paper, notes that AVERY and his colleagues also showed great caution in their conclusions about the genetic role of DNA. And JUDSON tells us the reason this happened: nucleic acids were then believed to be made up of a monotonous series of identical tetranucleotides and thus were incapable of any variety or specificity. They couldn't possibly carry the genetic information. It was only when ERWIN CHARGAFF developed a method for accurate quantitative analysis of the nucleotide content of DNA, that this mistaken view was corrected. CHARGAFF (1950) showed that the four kinds of nucleotides did not have to be present in equimolar amounts and that the DNA of each species had its own nucleotide ratio. JUDSON makes a convincing case that it was this finding that liberated us to think of genes made of DNA. He does not discuss the earlier evidence of genetic activity of DNA that came from the studies of the action spectrum of UV-induced mutation, but he would certainly have made the same argument to explain the modesty of the conclusions of the authors of that work.

JUDSON points out that even for proteins our imagination was limited by the prevailing chemical theory that amino acid sequences were restricted and repetitive because of structural constraints. JUDSON maintains that it was only when SANGER determined the amino acid sequence of bovine insulin and found no sequence restrictions (SANGER and TUPPY 1951) that theorists were liberated to think about a genetic code: sequence equals information.

My own limited recollection of the viewpoint of biologists in the 1940s differs from JUDSON on this point. Biologists of that time didn't know much chemistry and didn't think about the implications of a limit to variability in the primary sequence of proteins. We were much more impressed by their *biological* properties. We knew that, both as enzymes and as antibodies, proteins showed the capacity for amazing diversity, so why not as genes? Besides, we were not then so concerned about the need for a unique *sequence* in every gene. The idea that genetic information comes in a one-dimensional

array came to most of us only with the WATSON and CRICK (1953) paper. Before 1953, we could entertain two- and three-dimensional models for gene structures of head-spinning complexity.

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