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Perspectives

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The Amber Mutants of Phage T4

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A MAJOR effort of today's biology is the analysis of development by genetic methods, an approach so successful that previously unimaginable insights have become commonplace. A major moment in the growth of these studies was the discovery and analysis by DICK EPSTEIN and his colleagues of the amber mutants of phage T4.

The discovery of the ambers has been touched upon elsewhere (EDGAR 1966). The present history is a fuller account, offering perspectives not previously detailed. The story is very much of the Rochester T4 Group, headed by GUS DOERMANN, during the period from 1953 to the early 1960s. GUS's group was deeply involved in phage radiobiology, a discipline whose practitioners hurled poorly characterized reagents at invisible targets and hoped for interpretable responses.

LURIA (1947) and LURIA and DULBECCO (1949) observed that bacterial cells infected by more than one UV-irradiated phage particle produced a burst of viable progeny phage with a higher probability than expected if the irradiated phage survived independently of each other. This "multiplicity reactivation" demonstrated that irradiated phage particles could cooperate to come back to life. LURIA and DULBECCO offered a simple, well defined hypothesis for the phenomenon. They proposed that T2 phage (a close relative of T4) are made of functionally distinct subunits of equal UV sensitivity. A phage particle is "killed" by UV when any one of its subunits is "hit." An infected cell will produce progeny phage if, among the several infecting particles, there is at least one un-hit subunit of each type.

DULBECCO (1952) later recognized that a "critical test" of the subunit hypothesis required data collected at higher UV doses. At such doses, the model required that the survival curve for multiply infected cells (multicomplexes) become exponential, with a slope the same as that seen for singly infected cells (monocomplexes). He noted that the survival curve for multicomplexes did become exponential at high dose, but the slope was only about 0.2 of that seen with monocomplexes. Accordingly, DULBECCO rejected the uniform-sensitivity subunit model. In 1956, HARM did similar experiments with the related phage T4 and showed that the high-dose slope of multicomplexes was 0.4 of that for monocomplexes.

BARRICELLI (1956; see HARM 1956) proposed that the subunit theory for multiplicity reactivation be modified such that part of the phage was composed of largish subunits ("vulnerable centers"), while the remainder of the phage was composed of many small subunits. The sensitivity of a single infecting phage would be a measure of hits anywhere in it. Since one would rarely hit all copies of any given small subunit in any multicomplex, the survival of the multicomplexes would be determined primarily by the vulnerable centers. The quasi-final slope of the multicomplex survival curve would tell the fraction of a phage particle that was composed of vulnerable centers, while the shape of the shoulder of the curve would provide a count of the number of vulnerable centers. By this analysis, 40% of T4 was composed of about three vulnerable centers.

Beginning about 1952, DOERMANN and MARTHA CHASE, working with T4, conducted crosses between single particles of UV-irradiated phage and several particles of genetically marked, nonirradiated phage ("cross-reactivation experiments"). They found that the fraction of multicomplexes that produced phage did not fall with increasing UV doses, but that the probability that any given genetic marker from the irradiated parent would appear in the progeny did decrease. Analysis of these data showed that markers, unless very close to each other, were independently eliminated from the yields of the individual multicomplexes (DOERMANN *et al.* 1955). DOERMANN hoped that this kind of "probing" would help him in his efforts to get a fuller description of the T4 genome than was possible using the few plaque-morphology mutants that were available. He hoped that UV lesions would serve as generic markers that could be placed at high density throughout the genome. The problem, of course, was that the placing of these lesions was certain to be different from particle to particle, limiting their usefulness as "markers."

SEYMOUR BENZER (1955) used the *rII* mutants of T4 to examine genetic fine structure, exploiting the inabil-

ity of *rII* mutants to grow on λ lysogens, which do support growth of wild-type T4. DOERMANN exploited the selective-growth property of these mutants to extend his cross-reactivation analysis to high doses. He found that a wild-type allele of any conventional point mutation *rII* marker was "knocked out" (*i.e.*, not transmitted into a live phage particle) at high doses with a sensitivity that was 1/180 of that of the plaque-forming ability of a phage particle (DOERMANN 1961). This high resistance of individual markers suggested that the bit of genome transferred from a UV-killed phage to an unirradiated coinfecting phage in an individual act of "cross-reactivation" could be as small as 0.0056 of the genome.

In genetically mixed infections of λ lysogens, the wild type is dominant and phage are produced. DAVE KRIEG, a student in the DOERMANN group, exploited this dominance of the rII^+ allele to assess the sensitivity of gene functions to UV inactivation (KRIEG 1959). He UV-irradiated wild-type T4 and adsorbed them to a λ lysogen at low multiplicity along with several particles of *rII* mutant phage. He plated the complexes before lysis on a host that was permissive for both *rII* and rII^+ phage in order to measure, as a function of UV dose, the fraction of mixedly infected cells that could produce phage. KRIEG found that the UV sensitivity for the *rIIA* function was 10% of the sensitivity of the plaque-forming ability of T4, while for the smaller *rIIB* cistron the sensitivity was 5% of the plaque-forming ability. These values were comparable to the estimated sensitivity for a vulnerable center (40%/3 ≈ 13%). Other experiments by KRIEG had shown that, for phage production in the lysogen, the rII^+ function must be provided early in infection.

Putting KRIEG's radiobiological analysis of gene function together with DOERMANN and CHASE's cross-reactivation experiments provided a semi-molecular model for multiplicity reactivation. This model supposed that vulnerable centers are genes specifying early functions that must be expressed before the onset of genetic recombination, which in T4 is so frequent that one damage-free chromosome can almost always be assembled from damaged ones as long as the functions for doing it have survived (BARRICELLI 1956). This view received support from the thesis work of DICK EPSTEIN (1958), also a student in DOERMANN's group. DICK conducted multiplicity reactivation experiments in which infection was made by a mixture of two genetically marked parents. Qualitatively, an expectation of the model was realized: at high dose, each productive cell gave a burst composed primarily of one genotype, which was often recombinant for the markers employed. More on EPSTEIN's work later.

BOB EDGAR, who, as a student with DOERMANN, had identified localized negative interference in T4, arrived as a postdoc in MAX DELBRÜCK's Caltech lab shortly before I (also a DOERMANN student) left my postdoc spot at Caltech for a faculty job in Missouri. EPSTEIN arrived at Caltech soon thereafter, and in late 1959 visited us in Eugene, where I had landed after fleeing from Missouri.

During the Oregon visit, EPSTEIN and I discussed the state of UV radiobiology in T4 and identified a paradox. As mentioned above, *rII* gene function is required in λ lysogens but not in nonlysogens. The lysogen in standard use in GUS's lab was the *Escherichia coli* K12 derivative K12S(λ). The nonlysogen was *E. coli* B, the standard host for T-phage experiments. From KRIEG's work, described above, the *rII* gene functions appear to be vulnerable centers in K12S(λ). As part of his thesis work, EPSTEIN had carried out multiplicity reactivation experiments in that strain, comparing the survival of wild-type T4 multicomplexes with multicomplexes made of a complementing mixture of *rIIA* and *rIIB* mutants. These experiments supported the view that the *rII* genes act as vulnerable centers in K12S(λ). The argument underlying that conclusion was laid out in a review I wrote while at Caltech (STAHL 1959). However, the *rII* genes should not act as vulnerable centers in strain B, because null mutants of *rII* grow well in strain B. During his visit to Eugene, EPSTEIN remarked that his recent experiments showed that the multiplicity reactivation curves for T4 in those two hosts were not distinguishable. They should have been! The requirement for *rII* function in K12S(λ) should have increased both the high-dose slope of the multiplicity reactivation curve and the estimate of the number of vulnerable centers. We realized that the paradox could be resolved by proposing that T4 had two genes whose functions were required in B but not in K12S(λ), and that the functions of these two genes were about as UV-sensitive as were the functions of the *rII* genes.

Eventually, EPSTEIN returned from Oregon to Caltech, where he shared an apartment with graduate student CHARLEY STEINBERG. Referring to an event in early 1960, CHARLEY (personal communication) writes, "Dick brought up the *rII* mirror gene hypothesis several times, and I was not enamored of it. I just found it difficult to take radiobiology that seriously. . . . One evening at supper, with wine, he brought the hypothesis up yet again . . . and I said with considerable irritation, 'Dick, you don't believe that cockamamie idea any more than I do. If you did, you would have long ago started to hunt for mutants in those genes. You don't do it because you know you won't find any mutants.' Dick was taken aback by my fury and said that he would do it that very night after supper. I felt morally obliged to help him . . ."

DICK writes (personal communication), "Charley, of course, was an essential partner, but I do not remember his encouragement to do the experiments as being angry and impatient. It isn't Charley's style [and] he was agreeable to picking 2000 plaques in the first try. . . . We managed . . . to convince Harris [BERNSTEIN,

Caltech graduate student] to help us and offered the dubious reward of naming the mutants after him. Harris . . . had the nickname Immer Wieder Bernstein (*i.e.*, Forever Amber) . . ."

That night, several apparent B-specific mutants were isolated ("amber" mutants, of course). However, additional mutant isolations plus complementation tests revealed about 20 genes rather than the two that were anticipated. CHARLEY writes, "When I told Max [DELBRÜCK] about all the genes we were finding, his response was 'How dull!' "

Obviously, the original motivation for looking for B-specific genes was no longer useful, but the reality of an abundance of B-specific mutations was now undeniable. DICK writes, ". . . we fairly quickly grasped that the mutants might open the way to a characterization of the genes of T4, and some primitive physiological studies . . . were among our first efforts . . ."

BOB EDGAR (personal communication) writes, "[When I heard of DICK's mutants], I was filled with envy and wanted my own genes. So [I looked for and] found the [temperature-sensitive mutants of T4]. I was led to that during a conversation with [ALLAN] Campbell at Cold Spring Harbor about his [host-defective mutants] and Dick's *ambers*, which led us to the notion of conditional lethals . . ." EPSTEIN writes (personal communication) that it was through JEAN WEIGLE that the Caltech group became aware of the possible relevance of CAMPBELL's work to the understanding of the ambers. CAMPBELL, who was DOERMANN's successor at Rochester, has reviewed (1993) the history of the host-defective (*hd*) mutants of λ and of his interactions with WEIGLE and the T4 group.

A satisfactory explanation for the specificity of the amber mutations was obtained by comparing the plating properties of these mutants with the plating properties of the *hd* (later called *sus*) mutants (CAMPBELL and BALBINDER 1958; CAMPBELL 1959, 1961) and of "ambivalent" *rII* mutants (BENZER and CHAMPE 1961). Those hosts that plated *hd* mutants and some of those λ lysogens on which ambivalent *rII* mutants made plaques also plated the *amber* mutants. Apparently, K12S(λ) and many other strains of *E. coli* could suppress the mutant phenotype of certain alleles of any gene (BRENNER and STRETTON 1964). *E. coli* B could not suppress the phenotypes of those alleles. BRENNER and STRETTON decreed that all such suppressible mutants be called *amber*. As envisioned (dimly) by YANOFSKY and ST. LAWRENCE (1960) and (more clearly) by BENZER and CHAMPE (1962), the subsequent identification of chain-termination triplets and of mutant tRNAs that can read those triplets as if they stood for certain amino acids provided a satisfying molecular explanation for these suppressible mutants.

EPSTEIN, in the meantime, had moved to UCLA, where he undertook studies on the function of his various mutants in collaboration with two students, HILLARD BERGER and FRED EISERLING, and with LURIA and MARIE-LOUISE DIRKSEN at MIT. EPSTEIN soon after moved to Geneva, where he continued studies to determine the stage in the life cycle at which each of his amber mutants was blocked. EDOUARD KELLENBERGER, who made possible the early electron microscope studies of amber-infected cells, soon exploited the mutations for the analysis of particle morphogenesis. At Caltech, EDGAR and BILL WOOD later conducted such studies *in vitro*, with results that opened the way to the analysis of complex assembly pathways using *in vitro* complementation. In Geneva, BEN HALL, PETER GEIDUSCHEK, BRUCE ALBERTS, and others were influential in initiating new biochemical studies of the mutants. For PETER and for BRUCE, contact with the amber mutants led to career investigations of T4 transcription and DNA replication, respectively.

EPSTEIN's and EDGAR's parallel studies on ambers and *ts* mutants became more intense when BOB discovered that his *ts* mutants were, for the most part, in the genes that were identified by DICK's ambers. At the 1963 Cold Spring Harbor Symposium, the paper by DICK EPSTEIN, TOINON BOLLE, CHARLEY STEINBERG, EDOUARD KELLENBERGER, E. BOY DE LA TOUR, R. CHEVALLEY (Geneva), and BOB EDGAR, MILLARD SUSMAN, GETTA DENHARDT and ALEX LIELAUSIS (Pasadena) introduced the world to the awesome power of conditional-lethal mutations (EPSTEIN *et al.* 1964). The appearance of this publication implied that it was plausible to undertake a complete developmental analysis of a sophisticated biological system.

The amber mutants and their *ts* cousins, found by graduates of the DOERMANN group at Rochester as spin-off from their radiobiological analyses, provided the phage group with generic, genome-wide markers that could do for phage genetics what random radiation damages could never accomplish (and what RFLPs and SSRs now accomplish for human genetics). They provided a convincing demonstration of the circularity and dimension of the T4 linkage map (STAHL *et al.* 1964; STREISINGER *et al.* 1964), revealed the remarkable clustering of its genes according to function (EPSTEIN *et al.* 1964), and provided the material for an elegant demonstration of the colinearity of a gene and its polypeptide product (SARABHAI *et al.* 1964). More importantly, the steadfast pursuit of an explanation for multiplicity reactivation led to the discovery of mutants that freed the phage field from the genetic and radiobiological formalisms of the time by opening the door to studies of development that employed direct means for analyzing gene function.

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