

Amber Mutants of Bacteriophage T4D: Their Isolation and Genetic Characterization¹

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Introduction by Franklin W. Stahl³

Fifty Years Overdue

DICK Epstein and Bob Edgar, both with biology degrees in T4 genetics from the University of Rochester (Gus Doermann, advisor), pursued postdoctoral studies contemporaneously in Max Delbrück's lab at Caltech. T4 phage was (and is) such a beautiful object for molecular investigations that they continued working with it, attracting colleagues, including Charley Steinberg (Wu and Lindahl 2001), as they did so. Their work with T4 went stratospheric with the discovery that most of the essential genes of T4 could be identified by mutation to states that were lethal under some conditions but viable under others ("conditional lethal" mutants). [For particulars of this serendipitous realization, see Edgar and Epstein (1965), Edgar (1966), Stahl (1995), and Edgar (2004).] Armed with libraries of such mutants, and with the collaboration of visiting electron microscopist Edward Kellenberger and others, the Rochester-

ians famously demonstrated that phage development—at the molecular level—was now open for business (Epstein *et al.* 1964). That group effort was one of a number of papers that referred to work ("in press," "unpublished," or "in contemplation") describing the birth particulars of the amber mutants of Doermann's T4 strain T4D. The unpublished paper, which should have been the official "birth certificate" of these versatile mutants, has remained unpublished for more than 50 years after its conception. We have submitted the paper (Epstein *et al.* 2012) now, not only for its historic interest but also because no one knows when a properly registered certificate of birth may become a matter of importance. The authors apparently anticipated that the article would appear as the first of a series on the ambers and their uses. We may speculate on why it was not.

Dick shared his thoughts (and his mutants) freely—he was a devotee of the Delbrückian view that science was a group effort. Writing papers? Not so much. In 1951, Dick explained to me that Don Charles, his first-year advisor at Rochester, was a very bright guy who rarely published because he lost interest in a project as soon as he knew the answer. Does that explain why the text of Epstein *et al.* (2012) stayed in Dick's filing cabinet until it was retrieved by his colleagues following Dick's death (March 6, 2011)? Probably so.

The T4 world had put Dick's amber mutants to work (Wiberg *et al.* 1962) before the birth certificate was ready for publication. When that reality was combined with the spectacular 1963 Cold Spring Harbor presentation (Epstein *et al.* 1964) and the comprehensive GENETICS paper (Edgar *et al.* 1964), Dick likely looked upon a birth certificate as an uninteresting formality. (The ambers have arrived, haven't they? What more could one want?) Those papers were soon followed by a cornucopia of classic

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¹Minor corrections and editorial changes to the original typed manuscript were made by Costa Georgopoulos² (University of Utah) and Dominique Belin² (University of Geneva), who wrote the figure legends. For example, "phage" was systematically replaced by "bacteriophage." Unfortunately, the three tables and one of the figures that are mentioned in the article were not recovered in R. H. Epstein's archives. The map of Figure 2 was drawn by pencil at an unknown date. Since the last-discovered version of the article must have been written after 1964, the total number of complementation groups was set at 56, according to Edgar and Epstein (1965). One of the mutants shown in Figure 2, *amH17*, maps to gene 52. Frank Stahl (University of Oregon) rectified punctuation and grammar and formatted the article for the GENETICS of the 21st century.

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“amber papers” from various labs, demonstrating, for example, circularity of the T4 linkage map, clustering of genes by function, programs of viral development, roles of chain-terminating codons, colinearity of gene and protein product, self-assembly of organelles, mechanisms of DNA replication and its coupling with genetic recombination, and control of gene expression at the translational level. In the face of such a tide of fundamental advances in molecular genetics, the confirming of references, editing, writing a cover letter, and submitting the intended birth certificate must have seemed quite unnecessary and all too boring.

The belated publication by GENETICS of a famously overdue article confirms, in a formal way, the legitimacy of the ambers. In clerical detail, it tells how the mutants were induced, isolated, mapped, and sorted into complementation groups. With a few exceptions, the language and style is that of the era in which it was written. However, there has been some copy editing, presumably like that that would have occurred in the normal course of publication. Its proper place in the literature would have been as a companion paper with Edgar and Lielausis (1964) and Edgar *et al.* (1964), all three of which would likely have been dated a year or two earlier if progress had been orderly. Read it, and appreciate a time and a man.

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