

This Week's Citation Classic®

Peacock W J, Brutlag D, Goldring E, Appels R, Hinton C W & Lindsley D L.

The organization of highly repeated DNA sequences in *Drosophila melanogaster* chromosomes. *Cold Spring Harbor Symp.* 38:405-16, 1973.

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These studies were unique because they provided a molecular description of a large (approximately 20 percent) fraction of a eukaryote genome before DNA cloning technology was developed. The data provided a structural basis for considering the complex biological effects associated with heterochromatin, the part of the genome where highly repetitive DNA is located. In addition to this conceptual contribution, the work provided a technical advance in handling large quantities of *Drosophila* embryos, as required for biochemical work. [The SCI® indicates that this paper has been cited in over 150 publications.]

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The long-standing significance of the *Cold Spring Harbor Symposium* paper is in the baseline it provided for the analysis of a significant portion of a eukaryote genome. It was carried out in the pre-cloning days, and yet the results have remained essentially unchanged because of the exploitation of the special biochemical features of the class of DNA that we were investigating. The DNA analysed was isolated from *Drosophila melanogaster* embryos and included mitochondrial DNA and a number of homogeneous, highly repetitive sequences from nuclear DNA.

I initiated the work while on sabbatical leave at Stanford in 1971, with D. Hogness. During this period, a major problem of dealing with the biochemical analysis of *Drosophila* was solved, namely, the mass culturing of the flies. The system then worked out has since been increased in size and efficiency and still forms the basis for isolating a range of enzymes, as well as DNA, from embryos. At this time, the first of the highly repetitive sequences was isolated in silver nitrate/caesium sulphate gradients.

In 1972 Doug Brutlag (a graduate student of Kornberg), Elizabeth Goldring, and Rudi Appels (both Australian research workers) joined me in Canberra in analysing *D. melanogaster* DNA. A major breakthrough at this

stage was the exploitation of DNA-binding antibiotics in caesium chloride gradients to separate a series of homogeneous satellite DNAs, each of which proved to contain very simple DNA sequences tandemly repeated many thousands of times. The chromosomal analysis of the sequences was stimulated by C. Hinton and D.L. Lindsley, who visited Canberra in 1972-1973.

A specific problem that was addressed was the identification of the mitochondrial DNA as a specific entity and its characterization, identifying an unusual region of very high AT content. Analyses of the nuclear, highly repeated satellite species of DNA included demonstration of homogeneity of sequence by single-strand equilibrium gradient centrifugations, reassociation analyses, and pyrimidine tract analyses. The nucleotide sequences of the short repeats were determined by RNA sequence analysis. The study was important in establishing that highly repeated DNA was of several different sequence types, each being in relatively few long tandem arrays in the genome and that these blocks of repeated-sequence DNA were mostly situated in the heterochromatic regions of the chromosomes. We were able, by *in situ* hybridization techniques, to produce the first maps of the location of the various blocks of repeats on the chromosome complement of *Drosophila*.

This paper established many of the basic properties of highly repeated-sequence DNA that apply to most eukaryote organisms, including plants as well as animals. The paper also demonstrated that the highly repeated class of DNA was, in large part, responsible for the circular DNA molecules described by Lee and Thomas following exonuclease III resection of total nuclear DNA. The study complemented work done by the Hogness laboratory that also showed that the exonuclease-generated circles were largely derived from the satellite DNA species. These were important results for the understanding of genome organization in eukaryotes that, in the early 1970s, was still not well understood.

(Some key references are cited below.)¹⁻⁴

1. Gall J G, Cohen E H & Polan M L. Repetitive DNA sequences in *Drosophila*. *Chromosoma* 33:319-44, 1971. (Cited 330 times.)
2. Kram R, Botchan M & Hearst J E. Arrangement of the highly reiterated DNA sequences in the centric heterochromatin of *Drosophila melanogaster*. Evidence for interspersed spacer DNA. *J. Mol. Biol.* 64:103-17, 1972. (Cited 75 times.)
3. Laskowski M, Sr. The poly(dA-dT) of crab. *Prog. Nucl. Acid Res. Mol. Biol.* 12:161-88, 1971.
4. Hilliker A J & Appels R. Pleiotropic effects associated with the deletion of heterochromatin surrounding rDNA on the X chromosome of *Drosophila*. *Chromosoma* 86:469-90, 1982.