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This Week's Citation Classic

Radloff R, Bauer W & Vinograd J. A dye-buoyant-density method for the detection and isolation of closed circular duplex DNA: the closed circular DNA in HeLa cells.
Proc. Nat. Acad. Sci. US **57**:1514-21, 1967.
[Norman W. Church Lab. for Chemical Biol., California Inst. Technol., Pasadena, CA]

A buoyant-density method for the isolation and detection of closed circular DNA is described. The method is based on the reduced binding of the intercalating dye, ethidium bromide, by closed circular DNA. In an application of this method we have found that HeLa cells contain, in addition to closed circular mitochondrial DNA of mean length, 4.81 microns, a heterogeneous group of smaller DNA molecules which vary in size from 0.2 to 3.5 microns and a paucidisperse group of multiples of the mitochondrial length. [The *SCI*® indicates that this paper has been cited over 845 times since 1967.]

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"During the 1960s, Jerome Vinograd and most of the students and postdoctoral fellows in his laboratory were studying intensively the biological and physical properties of the closed circular DNA found in the papovaviruses and in mitochondria. In 1963, I became a graduate student in that laboratory and William Bauer, now at the State University of New York at Stony Brook, also joined the laboratory as a student shortly thereafter. Initially, I worked on several projects dealing with the structure and properties of the closed circular double-stranded DNA of polyoma virus, a papovavirus, while he began working with the chemistry of the interaction between the dye, ethidium bromide, and DNA isolated from the papovavirus, simian virus 40(SV-40). His work showed that closed circular DNA forms a band at equilibrium in CsCl buoyant density gradients in the analytical ultracentrifuge at a greater density than does the linear DNA in the presence of ethidium bromide because less dye is bound by the closed circular DNA.

"In the latter part of 1966, we began working together, under the guidance of Vinograd, to see if we could develop a useful preparative procedure for the separation of closed circular from linear DNA in CsCl buoyant density gradients containing ethidium bromide. I initially attempted the experiments with purified closed circular and linear polyoma viral DNA and was successful in separating the two forms. Within several months, the method was extended to the separation of closed circular DNA from polyoma virus infected cells and from noninfected cells. The closed circular DNA from noninfected HeLa cells was found to consist primarily of molecules the size of mitochondrial DNA. In addition, however, two other groups of circular molecules were found at low frequency. One group was a heterogeneous population of molecules which vary in size but are smaller than the mitochondrial DNA, while the other group contained molecules which were multiples of the mitochondrial length. The significance of the closed circular DNA larger and smaller than mitochondrial DNA is still not fully understood.

"I believe this paper has been cited frequently for two reasons. One is that it was published near the beginning of a series of a great many experiments with the closed circular DNAs found in viruses, mitochondria, and bacteria. A second reason is that it contains a simple and reliable method that provides good separation for closed circular DNA molecules from linear DNA molecules.

"Many aspects of this research remain in my memory. What stands out most clearly is that nearly every experiment tried was successful. That was an exciting experience and one which I believe occurs infrequently for most scientists.

"Vinograd died in 1976. He was instrumental in the development of a number of well-known techniques in centrifugation and in the elucidation of the structure of closed circular DNA in addition to his other research accomplishments. All who knew or worked with him admired and respected his intuition, his abilities, and his patience. A review of this field was recently published by Bauer."[1]

1. **Bauer W R.** Structure and reactions of closed duplex DNA. *Annu Rev Biophys. Bioeng.* **7**:287-313, 1978.