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This Week's Citation Classic

Hewish D R & Burgoyne L A. Chromatin sub-structure. The digestion of chromatin DNA at regularly spaced sites by a nuclear deoxyribonuclease. *Biochem. Biophys. Res. Commun.* **52**:504-10, 1973.
[Sch. Biological Sciences, Flinders Univ. South Australia, Bedford Park, South Australia]

When rat liver cell nuclei were incubated such that an endogenous deoxyribonuclease digested the nuclear DNA, the DNA products were found to have a regular series of discrete sizes. The deoxyribonuclease digested purified DNA in a random manner, and it was concluded that proteins within the nuclei restricted the access of the nuclease to regularly spaced sites on the nuclear DNA. [The *SCI*® indicates that this paper has been cited over 655 times since 1973.]

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"In 1973, when we published our evidence for a repeating substructure within the cell nucleus, researchers in the field of chromatin structure were eager for any new technique which would give insight into the structure of the DNA-protein complex. Several laboratories were apparently nearing the point at which they could have carried out our experiments. It is perhaps ironic, however, that although our research was not directed toward an investigation of chromatin substructure, we were uniquely well placed to carry out such a study.

"For some years, members of our laboratory had been studying the properties of isolated rat liver cell nuclei, in order to obtain an *in vitro* system for investigating DNA replication. One type of nuclear preparation was found to be unusual, in that the nuclei contained high levels of a deoxyribonuclease which rapidly digested the nuclear DNA when magnesium and traces of calcium ions were present.[1] Obviously, the nuclei were of little value for the study of DNA synthesis, as they underwent autodestruction under the very conditions necessary for DNA synthesis. It was noticed that, although the DNA was extensively degraded, very small fragments were not produced. We reasoned that the deoxyribonuclease must have some function in the intact cell, and I spent some time studying its properties, with few results of interest. As I was nearing the end of my PhD project, the time came to review the work and to decide whether the preparation was worth further investigation. In the course of one discussion, we decided to analyse the fragments of DNA produced by the enzyme in intact nuclei. To be honest, our recollections of our exact motives are hazy, but we were influenced by a previous publication of Williamson,[2] who had described a regular series of size classes among DNA fragments in the cytoplasm of cultured cells. We had wondered for some time whether the breakdown of the nuclear DNA in our system was in any way related to the production of those cytoplasmic fragments.

"Accordingly, I allowed a batch of nuclei to auto-digest for varying times, purified the DNA, and separated the fragments, by electrophoresis, on the basis of size. The results were dramatic. A series of discrete fragments was produced, indicating that the enzyme was limited in its action to certain sites on the chromatin and we therefore had the first evidence for a regular substructure within the nuclear chromatin. We proposed that the nuclear proteins were responsible for this regular structure, a supposition which has been shown to be correct.

"The reason for the large number of citations is clear. The technique which we introduced for probing the structure of chromatin was both very simple and effective. Other workers have since used different deoxyribonucleases to distinguish both lower and higher orders of chromatin structure and the results have correlated with electron microscopic and X-ray diffraction studies to produce a consistent and detailed picture of the subunit organization of the eukaryote chromosome. For a recent review, see the *Annual Review of Biochemistry*."[3]

1. **Burgoyne L A., Waqar M A & Atkinson M R.** Calcium dependent priming of DNA synthesis in isolated rat liver nuclei. *Biochem. Biophys. Res. Commun.* **39**:254-9, 1970.
2. **Williamson R.** Properties of rapidly labelled deoxyribonuclease acid isolated from the cytoplasm of primary cultures of embryonic mouse liver cells. *J. Mol. Biol.* **51**:157-68, 1970.
3. **McGhee J D & Felsenfeld G.** Nucleosome structure. *Annu. Rev. Biochem.* **49**:1115-56, 1980.