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This Week's Citation Classic

Clewell D B & Helinski D R. Supercoiled circular DNA-protein complex in *Escherichia coli:* purification and induced conversion to an open circular DNA form. *Proc. Nat. Acad. Sci. US* **62**:1159-66, 1969. [Dept. Biology, Univ. California, San Diego, and Revelle Coll., La Jolla, CA]

The plasmid Co/E1 was isolated as a super-coiled DNA-protein 'relaxation' complex. Exposure to agents or conditions affecting protein structure triggered a nicking event which resulted in a conversion of the plasmid DNA to an open circular configuration. [The *SCI*® indicates that this paper has been cited in over 965 publications since 1969.]

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"During 1967-1970, I was a National Institutes of Health (National Cancer Institute) postdoctoral fellow in Don Helinski's laboratory in the biology department at the University of California, San Diego, in La Jolla. Don was one of the few people in the world at that time involved in physical analyses of bacterial plasmids, he and his co-workers had recently characterized Co/E1 and a derivative of F by electron microscopy.

"As an approach to examining factors associated with Co/E1 maintenance, we embarked on an effort to isolate plasmid-protein complexes by sedimentation of lysates through sucrose density gradients. We made use of a lysis procedure based on recent work reported by Godson and Sinsheimer[1] on the use of various detergent mixtures to bring about the lysis of *E. coli* for different purposes.

" 'Gentle' lysis of *E. coli* (*Col*E1) cells labeled with H-thymine was accomplished by exposing lysozyme-EDTA treated cells (suspended in a sucrose solution) to a mixture of Brij 58 and deoxycholate. A 25 min. centrifugation at 48,000 x g pelleted the majority of the cellular DNA; and when the supernatant ('cleared lysate') was fractionated on a 15 to 50 percent sucrose density gradient, we observed a prominent peak which contained Co/E1 DNA almost exclusively. Upon further analysis, this substance proved to sediment slightly faster (24S) than purified supercoiled 23S plasmid DNA; and when exposed to proteolytic enzymes the DNA shifted, surprisingly, to 17S. We were able to show that the 24S material consisted of a supercoiled DNA-protein complex; and agents or conditions known to affect protein structure (e.g., proteases, certain detergents, and heat) triggered a nicking event which gave rise to relaxed circular molecules.

"The initial observations were reported in the paper cited above; whereas a more thorough characterization of the 'relaxation complex' and its related proteins followed.[2,3] Interestingly, the amount of plasmid DNA that was present as a relaxation complex was highly influenced by the presence of glucose (and cAMP) in the medium and ranged from 30 to 80 percent.[4] Relaxation complexes were subsequently found in a number of plasmid systems, and later work in the laboratories of Joe In-selburg[5] and David Sherratt[6] showed that in the case of *Col*E1, the complex related to conjugative mobilization

"The paper is cited frequently for its plasmid purification protocol. Significant volumes of the low viscosity, plasmid-en-riched 'cleared-lysate' could be loaded directly into dye-buoyant gradients, affording a 'high-yield' purification step in the preparation *of* covalently closed circular (CCC) molecules. In the case of some plasmids (e.g., Co/E1), however, this approach allows for the purification of only non-complexed CCC molecules; relaxation complex 'relaxes' under these conditions, and the DNA-protein complex ends up on the lower density side (shoulder) of the chromosomal band."

1. **Godson G N & Sinsheimer R L.** Lysis of *Escherichia coli* with a neutral detergent. *Biochim. Biophys. Acta* 149:476-88. 1967.
2. **Clewell D B & Helinski D R.** Properties of a supercoiled deoxyribonucleic acid-protein relaxation complex and strand specificity of the relaxation event. *Biochemistry* 9:4428-40, 1970.
3. **Helinski D R, Lovett M A. Williams P H, Katz L, Collins], Kupersztoch-Portnoy Y, Sato S, Leavitt R W, Sparks R, Herishfield V, Gulney D G & Blair D G.** Modes of plasmid DNA replication in *Escherichia coli.* (Goutian M & Hanawalt P. eds.) *DNA synthesis and its regulation.* Menlo Park. CA: Benjamin, 1975. p. 514-36.
4. **Clewell D B & Helinski D R.** Effect of growth conditions on the formation of the relaxation complex of supercoiled *Col*El deoxyribonucleic acid and protein in *Escherichia coli. J. Bacteriology* 110:1135-46. 1972.
5. **Inselburg J.** Studies of colicin El plasmid functions by analysis of deletions and TnA insertions of the plasmid. *J. Bacteriology* 132:332-40. 1977.
6. **Warren G J, Twigg A J & Sherratt D J.** *Col*El plasmid mobility and relaxation complex. *Nature* 274:259-61. 1978.