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Clewell D B. Nature of Col E_1 plasmid replication in *Escherichia coli* in the presence of chloramphenicol. *J. Bacteriology* 110:667-76, 1972.
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The plasmid Col E_1 in Escherichia coli was found to replicate extensively when protein synthesis was inhibited by exposure of cells to chloramphenicol. During the first two to four hours there was an exponential increase in plasmid DNA while chromosomal replication was shut down. Over a period of 10 hours plasmid DNA increased from 24 to 3,000 copies per cell. [The SCI® indicates that this paper has been cited in over 1,075 publications.]

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In 1970 I joined the faculty at the University of Michigan as an assistant professor after having spent three years as a postdoctoral fellow in Don Helinski's laboratory at the University of California, San Diego (UCSD). At UCSD I had been involved in analyses of the plasmid Col E_1 in *Escherichia coli* and was successful in isolating a plasmid DNA-protein complex[1] that we referred to as "relaxation complex." The supercoiled plasmid could be triggered *in vitro* to undergo a protein-promoted nick that resulted in a relaxation of the DNA to an open circular configuration. The complex was later shown to relate to the ability of the plasmid to be conjugatively mobilized.[2,3] We also showed that when cells were exposed to chloramphenicol (a protein synthesis inhibitor) the amount of relaxation complex remained constant while noncomplexed plasmid DNA accumulated.[4]

Upon moving to Michigan, I initially focused on a characterization of Col E_1 replication in the presence of chloramphenicol with the idea that such a system might be useful in studying the effects of various inhibitors of DNA replication. The above-cited paper chronicles these efforts. A somewhat surprising result concerned the extent to which replication occurred under these conditions. Whereas the synthesis of chromosomal DNA shut down relatively early during chloramphenicol exposure, plasmid DNA actually increased exponentially for two to four hours, after which a maximum rate was attained. Replication continued for as long as 10 hours in the presence of the drug, during which time the plasmid content went from about 24 to about 3,000 copies per cell. The rate of replication and total accumulation of plasmid DNA depended somewhat upon the medium being used.

The system subsequently proved valuable in analyzing the effects of various chemical agents. Most significant was the observation that rifampin dramatically inhibited replication.[5] This was at first a real mystery, since at that time rifampin was known to inhibit RNA polymerase but not to directly affect DNA replication. The importance of RNA in priming DNA synthesis, however, soon became evident in many systems. In the case of Col E_1, supercoiled plasmid DNA that had accumulated in the presence of chloramphenicol for more than four hours still contained significant amounts of unremoved RNA (used for priming), presumably because of a gradual breakdown in the repair (RNA removal) system during the lengthy inhibition of protein synthesis.[6]

The above-noted paper is frequently cited in connection with studies where investigators have desired to "amplify" specific plasmid chimeras.[7] Many of the commonly used cloning vectors are derivatives of, or are related to, Col E_1 and thus replicate extensively in the presence of chloramphenicol. Ironically, at the time the paper was submitted for publication, its value in this regard was totally unforeseen.

1. **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. USA* 62:1159-66, 1969. (Cited 10 times.)
2. **Inselburg J.** Studies of colicin E1 plasmid functions by analysis of deletions and TnA insertions of the plasmid. *J. Bacteriology* 132:332-40, 1977. (Cited 50 times.)
3. **Warren G J, Twigg A J & Sherratt D J.** Col E_1 plasmid mobility and relaxation complex. *Nature* 274:259-61, 1978. (Cited 75 times.)
4. **Clewell D B & Helinski D R.** Effect of growth conditions on the formation of the relaxation complex of supercoiled Col E_1 deoxyribonucleic acid and protein in *Escherichia coli*. *J. Bacteriology* 110:1135-46, 1972. (Cited 275 times.)
5. **Clewell D B, Evenchik B & Cranston J W.** Direct inhibition of Col E_1 plasmid DNA replication in *Escherichia coli* by rifampicin. *Nature New Biol.* 237:29-31, 1972. (Cited 80 times.)
6. **Blair D G, Sherratt D J, Clewell D B & Helinski D R.** Isolation of supercoiled colicinogenic factor E1 DNA sensitive to ribonuclease and alkali. *Proc. Nat. Acad. Sci. USA* 69:2518-22, 1972. (Cited 180 times.)
7. **Maniatis T, Fritsch E F & Sambrook J.** *Molecular cloning: a laboratory manual.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1982. 545 p. (Cited 13,250 times.)