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An effect of chloramphenicol on the incorporation of tritiated thymidine into chromosomal DNA

The inhibitive action of chloramphenicol on protein synthesis has been utilized extensively and with considerable success for the elucidation of the relationships between nucleic acids and proteins in microorganisms[1–3]. The apparent absence of a direct effect of this antibiotic on DNA replication in microorganisms[3], the probability that DNA in microorganisms is less intimately associated with a protein moiety than is chromosomal DNA[4,5], and the linear pattern of DNA increase in bacteria[6,7,8] as opposed to the stepwise pattern characteristic of DNA synthesis in chromosomal systems, have led us to the notion that chromosomal protein may play a controlling role in chromosomal DNA replication in addition to the structural function recently suggested[9,10]. We have explored this possibility by studying the incorporation of tritiated thymidine into meristematic cells of onion root tips in the presence and absence of chloramphenicol; the incorporation of [^{14}C]thymidine was studied under similar experimental conditions as a control for possible tritium effects[11].

Onion roots were immersed in a solution of 300 μg/ml chloramphenicol for periods of 6 and 24 h prior to the addition of labeled thymidine, with renewal of the chloramphenicol solutions every 6 h. [^{3}H]thymidine was used at a concentration of 2.5 μC/ml and a specific activity of 360 μC/μmole. The roots were incubated with the isotope for 11 h, fixed, briefly hydrolyzed, and squashed. Kodak AR-10 stripping film was applied and the preparations were exposed for 7 days. Assay of the [^{3}H]thymidine solutions before and after incorporation indicated that ample amounts of the tracer were available for incorporation throughout the incubation period.

The semi-quantitative analysis of the [^{3}H]thymidine-labeled preparations led to the following conclusions: (a) the percentage of cells with labeled nuclei did not differ significantly from control values in the root tips treated with chloramphenicol for either 6 h or 24 h; (b) the percentage of heavily labeled nuclei (nuclei with autoradiographic grain densities above the limit for reliable direct counting) was considerably higher in the treated roots than in controls. Instrumental analysis of the heavily labeled fraction of nuclei by means of a scanning microscope designed by GULLBERG[12] resulted in the data plotted in Fig. 1. The graph shows a progressive increase in the amount of [^{3}H]thymidine incorporation in nuclei of chloramphenicol-treated roots. The effect of the drug appears even more strikingly when we consider that the heavily

Abbreviation: DNA, deoxyribonucleic acid.

labeled fraction represents a larger proportion of the cells in treated than in control roots. Photometric DNA measurements showed an increase in DNA levels which could account for some, if not all, of the increased [^{3}H]thymidine incorporation in the presence of chloramphenicol. Further details of this analysis will be published elsewhere.

Very different results were obtained when [^{14}C]thymidine (0.5 μC/ml, 2.7 μC/mole) was substituted for the tritiated precursor: no significant incorporation differences were observed between either of the two chloramphenicol treatments and the controls. While the [^{3}H]thymidine-incorporation data thus show a chloramphenicol effect which suggests a functional role for protein in chromosomal DNA synthesis, the [^{14}C]thymidine results indicate that we are dealing primarily with an effect of tritiated thymidine on DNA which is amplified in the presence of chloramphenicol.

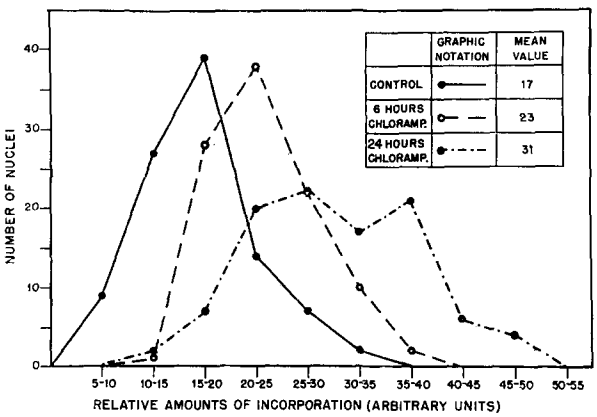

Fig. 1. The incorporation of [^{3}H]thymidine into meristematic nuclei of onion roots under the influence of chloramphenicol (300 μg/ml).

Further experiments, guided by the working hypothesis that normal chromosomal DNA replication requires the integrity of the DNA molecule as well as the presence of a proteinaceous stabilizing element, are in progress.

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