DNA REPLICATION AND THE CONSTRUCTION
OF THE CHROMOSOME

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It was previously proposed that the normal synthesis of DNA in higher cells was dependent on concurrent synthesis of histone. Evidence for this was based on the fact that four relatively specific experimental manipulations of the rate of histone synthesis led to characteristic alterations in the rate of DNA synthesis. A possible insight into the way histone might function during DNA replication came from the finding that added histone has the capacity to remove nascent DNA from a presumptive replication complex when tested in a cell-free system. In continuing these studies on the coupling between DNA synthesis and chromosome assembly, I will present further evidence that (a) nascent DNA is associated with a complex, possibly some "nuclear organelle", and (b) newly made histone enters the chromosome almost exclusively at the growing fork of DNA replication.

THE ASSOCIATION OF NASCENT DNA WITH A NUCLEAR COMPLEX

The basic assay to be used in this communication involves the treatment of isolated chick erythroblast nuclei with pancreatic DNase in the presence of Mg. Others have used analogous assays as a probe to the structure of nuclear chromatin. For pancreatic DNase, the digestibility of DNA in nuclei is a function of the dose of DNase treatment, and the extent to which histone covers the DNA.

Figure 1 shows the DNase sensitivity of newly-replicated DNA in isolated nuclei from pulse-labeled cells. The sensitivity is monitored as a function of replication time during a continuous exposure to 3H-TdR. Similar curves apply to chromatin. The data indicates that cell samples taken at 30 seconds after addition of label are

A. R. Kolber et al. (eds.), Mechanism and Regulation of DNA Replication
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II. EUCAVOTES—MECHANISM OF CHROMOSOME REPLICATION

FIGURE 1. Sensitivity of pulse-labeled DNA to DNase. Primitive chick erythroblasts were obtained from the blood islands of White Leghorn Hen eggs after 4 days of incubation at 37°C. The cells were incubated in vitro with H3-TdR (50 μc/ml; 15 Ci/mM) for increasing periods of time. Nuclei were obtained by suspending the washed cells in cold RSB (0.01M NaCl; 0.01M Tris-HCl, pH 7.2; 0.003M MgCl₂) containing 0.5% Nonidet P40. The nuclei were resuspended in 0.25M sucrose containing 5mM Na-phosphate buffer, pH 6.7 and 3mM MgCl₂. Nuclei (10⁶/ml) were digested with pancreatic DNase (Sigma) as described by Mirsky (1972). Digestion was at 22°C for 30 minutes at a DNase concentration of 20 μgm/ml. At the end of the digestion, the remaining DNA was precipitated with 5% TCA and carrier, washed and counted. All TCA precipitable counts could be recovered from the pelleted nuclei after digestion.

relatively resistant to DNase when compared to samples taken after longer exposures to label. As will be shown later, these in turn are much more resistant than free DNA. High DNase concentrations eventually digest over 90% of the resistant DNA labeled for 30 seconds. This indicates that the label is probably in DNA. It is un-