secondary and tertiary branches (Fig. 1b). It is postulated that branched structures (B) may determine specific interactions between DNA and protein.

In general, structure B, containing a few unpaired nucleotides, would be expected to be less stable than structure A. Protein, however, may stabilize structure B by association with the DNA, particularly if there is some specificity of interaction with the loop containing unpaired nucleotides. Such an association might be involved in the repression or activation of gene function. For example, a branched region of DNA containing the operator may be stabilized by repressors to block starting sequences for the synthesis of messenger RNA. It is conceivable that there are chemical modifications of unpaired nucleotides which help to shift the equilibrium toward conformation B, making an operator more readily accessible to repressors.

The model could possibly be applied to other problems. For example, some loops could be susceptible to specific enzymes such as DNases and methylating enzymes responsible for host-specific sensitivity and stability of DNA[4]. The spatial arrangement of DNA in chromosomes might partially derive from branched structures of DNA stabilized by proteins.

Experiments to test the hypothetical model can be based on the prediction that the branches contained in structure B should be formed by denatured, single-stranded DNA even without external stabilization. Some base-pairing in single-stranded denatured DNA has been demonstrated[5]. One might expect that denatured DNA would in some cases be more suited to detect the specific interaction with protein than native DNA.

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Ascorbate Stimulation of RNA Synthesis

INTEREST in the possible role of histone as an inhibitor of DNA dependent RNA synthesis has increased recently[1,2]. During an investigation of the synthesis of microgram quantities of RNA by wheat nuclei isolated by the method of Johnson et al.[3] it was observed that the addition of 10 mmolar ascorbate to the medium in which the nuclei were incubated resulted in a large increase in the amount of RNA synthesized (Table 1). No stimulation of DNA synthesis by ascorbate was ever observed even under conditions of active DNA synthesis.

Table 1. ASCORBATE STIMULATION OF RNA SYNTHESIS

	mμME RNA bases	Mean
Zero time controls (nuclei only)	25	25
Basic medium	58	67
	75	
"Ascorbate" medium	142	150
	157	

The nuclei were incubated in a basic medium containing 6 mmolar ATP, 10 mmolar ethyl carbamate, 10 mmolar glucose, 10 mmolar ribose, 10 mmolar RNA hydrolysate 1 mmolar magnesium sulphate, 50 mmolar phosphate buffer pH 6·8, 1 mmolar sucrose, and 0·5 mmolar calcium chloride. In addition the "ascorbate" nuclei had 10 mmolar ascorbic acid in their medium. All incubations were for 120 min at 30° C. The RNA was estimated by a modified Schmidt–Thannhauser method.

During incubation the nuclei remain morphologically unchanged, which was shown by microscopic examinations of samples stained in pyronine and methyl green, and even during periods of active protein synthesis very little protein is left in the medium when the nuclei are removed by centrifugation (unpublished results of W. O. James). When RNA synthesis is stimulated by ascorbate, up to 40 per cent of the nuclear protein is released into the medium.

I precipitated this protein with 5 per cent trichloroacetic acid, but it was soluble in dilute hydrochloric acid. About 50 per cent was precipitated at pH 10·6, which indicated that at least part of the released protein is histone. The protein was further analysed by two dimensional chromatography of the products of acid hydrolysis. With the solvent combination of butanol-acetic acid-water (8 : 1 : 1) followed by phenol ammonia[4], ten distinct ninhydrin positive spots were located in the total released protein and the protein soluble at pH 10·6. The fraction which was insoluble at pH 10·6 showed only nine spots, and the missing one had the same R_F as glutamate. Arginine[5] and proline[6] were identified by specific staining in all protein hydrolysates.

One of the major difficulties in the initiation of nucleic acid synthesis with isolated wheat embryo nuclei has been the apparent presence of control mechanisms within the nucleus[7]. Ascorbate does not remove these controls and when added to nuclei which are not synthesizing RNA it has no effect at all; neither nucleic acid synthesis nor the release of appreciable quantities of protein is induced. This is in agreement with the observations of Schopfer[8], who has concluded that ascorbate removes histone from inducible chromatin.

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Search for Radiation Deaminations in DNA

IT has been demonstrated that the free nucleic acid bases, adenine and cytosine, are deaminated in dilute aqueous solutions by ionizing radiation[1,2]. Neglecting ring destruction reactions, the deaminated analogues (hypoxanthine and uracil) are among the chief products of the radiolyses of these bases; this suggests a mechanism for mutagenesis induced by radiation. It is known that the dilute nitrous acid treatment of the free bases, adenine, guanine or cytosine, leads to the production of the corresponding hydroxyl compounds, hypoxanthine, xanthine and uracil, respectively[3,4]. Nitrous acid treatment of intact tobacco mosaic virus actually results in the formation of mutants[5]. In addition, alteration of the amino-acid sequence in the protein of the mutant virus has been demonstrated[6]. Deaminations induced by radiation should play the same part as deaminations induced by nitrous acid.

We decided to search for evidence of deamination induced by radiation in water solutions of intact DNA. To make the search as sensitive as possible, our work was carried out on DNA labelled with carbon-14. We looked for the appearance of the specific deaminated nucleotide, 5'-deoxyuridylic acid (5'-dUMP), from the 5'-deoxycytidylic acid (5'-dCMP) of the DNA, because the 5'-dUMP is available commercially for use as a carrier in chromatographic work. The other possible deaminated nucleotides from DNA, deoxyinosinic acid and deoxyribxanthine phosphate, are not commercially supplied. In addition cytosine gives a very high radiation yield of its deaminated analogue, uracil. It therefore appeared that our chances of finding deamination might be greatest in the transformation of deoxycytidylic acid to deoxyuridylic acid.

The labelled DNA was prepared by exposure of Chlorella to labelled carbon dioxide; hydrolysis of the algae (2 ml.