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The role of deoxyribonucleic acid in ^{32}P incorporation into isolated nuclei *

The importance of deoxyribonucleic acid (DNA) in the synthesis of nuclear protein and ribonucleic acid (RNA) has been demonstrated, but its action does not seem to be very specific[1,2]. Since different workers have observed the incorporation of various precursors into DNA in isolated nuclei[3–5], we have undertaken to clarify the role of pre-existing DNA in DNA synthesis with rabbit appendix nuclei.

Nuclei isolated from the appendix and Peyer's patch of adult rabbits by a slight modification of the method of ALLFREY *et al.*[1] were incubated with ^{32}P-orthophosphate according to FRIEDKIN AND WOOD[4]. The procedure of digestion with pancreatic deoxyribonuclease (DNase) and subsequent treatment of nuclei were essentially similar to the ones employed by ALLFREY *et al.*[1]. 1 ml of incubation mixture containing about $3 \cdot 10^8$ nuclei and 10 μC ^{32}P were shaken at 37° for 90 min. The acid-soluble organic-phosphate fraction (OASP) was obtained according to HOKIN AND HOKIN[6]. Nucleic acids were extensively purified by the routine method used in this laboratory[7]. Radioactivity incorporated into RNA and DNA was evenly distributed among various nucleotide fractions obtained chromatographically (Dowex-1, formic acid systems) following alkaline hydrol-

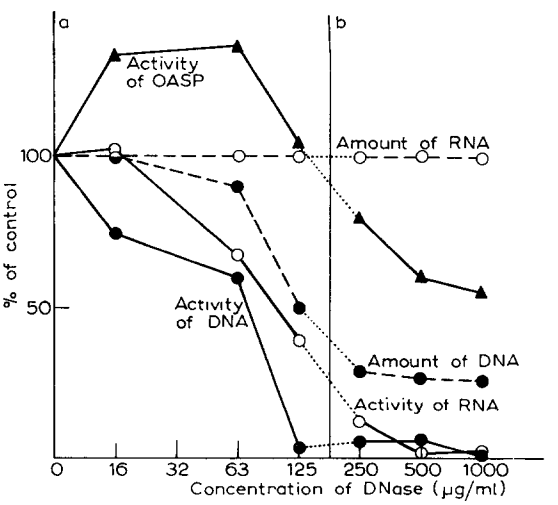

Fig. 1. The effect of DNase treatment of isolated nuclei. Activity refers to the total radioactivity (counts/min/tube). With nucleic acids, this was calculated from the specific activity (counts/min/μg P) of purified samples and the total amount (μg P/tube) determined by the conventional Schneider method. Specific activities of OASP, RNA and DNA of untreated control nuclei were 1076, 32, 2.4 (Expt. a) and 759, 43, 0.8 (Expt. b).

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ysis and DNase digestion, respectively, suggesting that the observed incorporation represents a synthesis of ribo- and deoxyribopolynucleotides.

As shown in Fig. 1, the removal of 70–80% of the DNA almost completely abolished the incorporation of ^{32}P into DNA and RNA. The incorporation into DNA was impaired more severely than that into RNA, while even after an exhaustive removal of DNA, incorporation into OASP was reduced by only 40%. Table I illustrate how these lost incorporation activities were restored by the addition of various acidic substances. Not only salmon-sperm DNA but also yeast RNA was effective. Except for a marked increase in the incorporation into OASP of control and digested nuclei, the effect of a mixture of 2',3'-mononucleotides was ambiguous, while chondroitin sulphate was as active as RNA. It is thus shown that at least the bulk of DNA present in cell nuclei is not necessary for ^{32}P incorporation into a fraction which behaves like DNA in our procedure. In nuclei treated with a higher concentration of DNase (1 mg/ml), however, the incorporation into DNA (but not into RNA) failed to show a significant restoration by the addition of either RNA or DNA. Chondroitin sulphate was inactive with these nuclei.

TABLE I

RESTORATION OF ^{32}P INCORPORATION INTO DNASE-TREATED NUCLEI

Expt. No.	*Nuclei*	*Additions*	*Total activity (% of control)*		
			OASP	*RNA*	*DNA*
I	Untreated	— (control)	100	100	100
		Yeast RNA	163	100	130
		Salmon-sperm DNA	76	88	72
	DNase-treated**	—	97	17	1
		Yeast RNA	135	98	57
		Salmon-sperm DNA	81	103	70
	Untreated	Chondroitin sulphate	107	115	97
		2',3'-mononucleotides	398	83	57
II	DNase-treated**	—	64	10	11
		Chondroitin sulphate	74	31	43
		Yeast RNA	114	30	33
		2',3'-mononucleotides	496	13	24

* 4 mg (Expt. I) or 2 mg (Expt. II) of respective compounds were added per tube.
** Concentration of DNase, 125 μg/ml. DNA removed, 64% (Expt. I) and 73% (Expt. II).

Experiments are now in progress to determine whether the nucleotide nature is not required for an acidic substance to effect a full restoration of the lost activity of DNase-treated nuclei.

Cytochemistry Laboratory, Yamaguti Medical School, Ube (Japan) MUTSUO SEKIGUCHI*
ATUHIRO SIBATANI

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* On leave from the Department of Biology, Faculty of Science, Osaka University, Osaka, Japan.