DIFFERENTIAL LABELLING OF DNA IN HIGHER PLANTS

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Summary. Under our experimental conditions labelling of DNA in higher plants with ^{32}P phosphate, ^{14}C uridine and ^{14}C thymidine shows two distinct species of labelled DNA : bulk-DNA and a satellite DNA. The bulk DNA (ρ = 1.696 g.cm^{-3}) does not incorporate ^{32}P phosphate, but ^{14}C thymidine. On the other hand the satellite-DNA (ρ = 1.720 g.cm^{-3}) does not incorporate ^{14}C thymidine, but ^{32}P phosphate. Both have ^{14}C-Uridine as a precursor. An attempt has been made to establish the cellular localisation of these two DNA's.

INTRODUCTION

It is widely known that the nucleus of eukaryotic cells contains only part of the cellular DNA. Organelles, notably the mitochondria and the chloroplasts, have their own DNA. Furthermore, studies indicate that nuclear DNA is heterogeneous.

Most of the nuclear satellite DNA's currently referred to, really belong in the category of ribosomal DNA. A few, notably among the mammalia, are still regarded as metabolic or messenger DNA (1, 2); others, among the higher plants seem to be involved in particular metabolic processes such as delayed replication or repair synthesis (3, 4).

Our purpose is to show that, by using radioactive precursors, two fractions can be distinguished in plant DNA and that they probably have distinctive processes of biosynthesis.

MATERIAL AND METHODS

Sterilized seeds of *Vicia faba, Zea mays* and *Raphanus sativus* germinate in the dark on filter paper imbibed with distilled water. Labellings are carried out on severed roots (broad bean and corn) or on whole plants (radish) for about 24 hrs. Samples, thoroughly washed with cold distilled water are gently ground in a 0.02 M tris buffer, pH 8, containing 0.4 M sucrose, 0.01 M NaCl, and 0.005 M EDTA. Cellular fractions result from differential centrifugation of the homogenate. The DNA is extracted fol-

lowing a procedure which closely parallels that of MARMUR (5). The DNA solutions are adjusted at an average density of 1.700 $g.cm^{-3}$ with CsCl and are centrifuged at equilibrium in a preparative ultracentrifuge (6). The fractions corresponding to each of the DNA's are pooled and dialysed. Their base composition is determined by hydrolysis in formic acid (7), while the nucleotide composition is obtained after enzymatic hydrolysis (8).

RESULTS

It can be ascertained from the sedimentation patterns of radioactive DNA, extracted from radish seedlings or from roots of broad bean or corn (fig. I), that the total DNA separates itself into two bands. The one (ρ = 1.696 $g.cm^{-3}$), which has the greater optical density is strongly radioactive after labelling with ^{14}C thymidine, but is weakly so when the samples incubate in ^{14}C uridine. Under such experimental conditions this fraction is never radioactive after incubation in ^{32}P phosphate. By contrast, the other band (ρ = 1.720 $g.cm^{-3}$), which represents between 5% and 10% of total DNA, is strongly radioactive when ^{14}C uridine or ^{32}P phosphate is used; its radioactivity is nil after incubation of samples in a ^{14}C thymidine solution. Under these experimental conditions then, ^{14}C thymidine and ^{32}P phosphate are the specific labellers of each of the two DNA fractions respectively.

Two hypotheses may account for this fact: either the two kinds of DNA molecules belong to the same subcellular fraction or they do not. To satisfy the first hypothesis, their biosynthetic processes would have to be different (at the level of precursors or polymerases for example). To satisfy the second, the exogeneous precursor would be used in only one of the two subcellular fractions.

We have therefore tried to determine the cellular localisation of these two fractions of DNA. Cellular fractionation experiments show (fig. 2) that the greater part of the ^{14}C thymidine labelled DNA is in a fraction, rich in nuclei, sedimenting at 3 000 g (table I). By contrast ^{32}P labelled DNA is present in both this fraction and in the fraction sedimenting at 27 000 g (fig. 2 and table I). Thus, the ^{14}C thymidine radioactivity represents nuclear DNA, and it follows that the ^{32}P labelled DNA might belong in the category of cytoplasmic DNA. However, analysis of the sedimentation patterns shows that the DNA of the 27 000 g fraction is at least partially sheared.

Electron microscopic examination of 3 000 g and 27 000 g pellets

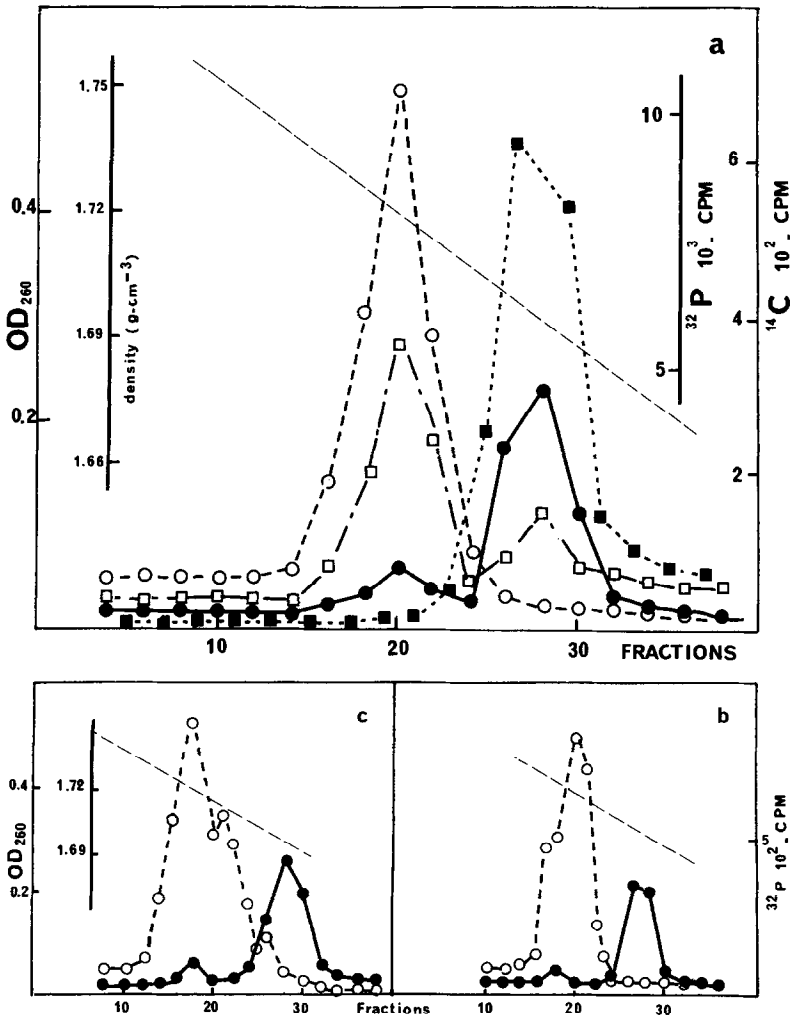

Figure I : CsCl sedimentation patterns of DNA from broad bean, corn and radish.

a) DNA from five day old broad bean root tips; labelling were for 24 hrs. with, either ^{32}P Phosphate, ^{14}C uridine or ^{14}C thymidine.

b) DNA from four day old corn root tips; labelling was for 24 hrs.

c) DNA from 48 hr old radish seedlings; labelling was for 4 hrs.

Each gradient contained about 200 µg of DNA. Centrifugations were achieved in a Spinco R.40 rotor at 33 000 rpm for 72 hrs. (—●—●—●) optical density, (○- -○- -○) ^{32}P radioactivity, (-□- -□- -□-) ^{14}C uridine radioactivity, (-■- -■- -■-) ^{14}C thymidine radioactivity.

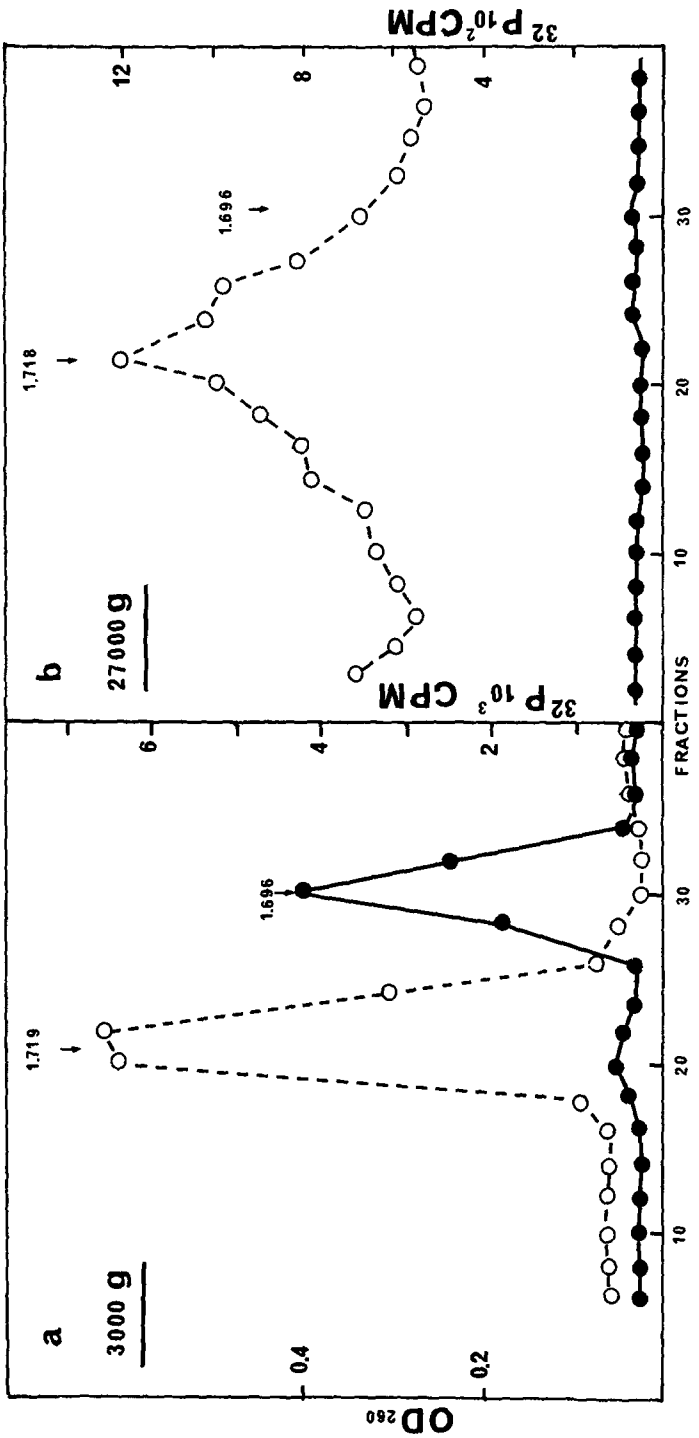

Figure II : CsCl sedimentation patterns of broad bean DNA extracted from a crude nuclear fraction (3 000 g pellet), (a), and from a crude cytoplasmic fraction (27 000 g pellet), (b). (—●—●—) optical density, (-○--○--○-) ^{32}P radioactivity. Labelling was for 24 hrs.

TABLE I

DNA radioactivity in the two subcellular fractions

	^{32}P Phosphate cpm	^{14}C Thymidine cpm
"nuclear fraction" 3 000 g. pellet	37 000	2 500
"cytoplasmic fraction" 27 000 g. pellet	22 000	300
Ratio $\frac{\text{cpm in 27 000 g pellet}}{\text{cpm in 3 000 g pellet}}$	0.59	0.12

Broad bean root tips were labelled for 24 hrs. with either ^{32}P phosphate or ^{14}C thymidine.

shows that the structures of numerous nuclei alter during the extraction procedure, and that it is possible for relatively important accumulations of chromatine to sediment at 27 000 g; so that the above-observed results have an explanation if we accept that the two fractions of DNA result from two categories of chromatin partially separated in the course of differential centrifugation.

Work has begun to determine which of these two hypotheses is valid.

Results of the radioactivity recovered after hydrolysis of the two DNA fractions appear on table II. It can be seen that ^{14}C uridine, the only precursor common to the two fractions is transformed "in vivo" into thymidylic and cytidylic nucleotides before being incorporated into the DNA. In higher plants, as in the microorganisms, the uridine is then quite clearly a precursor for pyrimidic nucleotides.

Rich in guanine and cytosine (table III) the satellite DNA is distinguishable from the bulk-DNA which is rich in adenine and thymine. Moreover, we note the abscence of 5-methylcytosine in this satellite.

DISCUSSION

For diverse methodological reasons, the numerous studies on the DNA

TABLE II

Recovered radioactive bases or nucleotides after hydrolysis of both DNA species following incorporation of different labelled precursors.

labelled precursor	DNA species	A or dAMP	G or dGMP	C or dCMP	mC dmCMP	T or TMP	U or UMP
^{32}P	Satellite	+	+	+	-	+	-
	bulk	-	-	-	-	-	-
^{14}C-uridine	Satellite	-	-	+	-	+	-
	bulk	-	-	+	+	+	-
^{14}C-thymidine	Satellite	-	-	-	-	-	-
	bulk	-	-	-	-	+	-

These results were achieved with broad bean root tips and radish seedlings. All pulse labellings were for 24 hrs., except for ^{32}P labelling of radish seedlings, which was for 4 hrs.

TABLE III

Nucleotide compositions of satellite and bulk DNA's of radish seedlings

	dAMP	dGMP	dCMP	dmCMP	dTMP	% G+C
satellite	21.1	26.4	32.6	-	19.8	59
bulk	30.9	19.1	16.2	2.9	30.9	38.2

Satellite DNA composition was determined by counting radioactive spots on the chromatograms, whereas bulk DNA composition was determined by measuring optical density of chromatogram eluates at the respective nucleotide maximum absorbancies.

of higher plants, do not yet make extensive use for radioactive labellers. However, as the work outlined above indicates, these can permit us to distinguish two DNA fractions. What biological significance does differential labelling of this kind have ? At present it is difficult to say. Yet, whatever the cellular localisation of the satellite DNA may be, it is clear that we must seek the explanation of this phenomenon, either in the per-

meability of diverse cellular compartments to a given precursor (thymidine for example), or in the distinctive enzymatic systems which are involved in the biological synthesis of these two types of molecules. PEARSON and INGLE (9) have recently considered the possibility that the dense satellite DNA's observed in plants, notably after "stress", could have a bacterial origin. In our view this does not seem to accord with the labelling properties of satellite DNA's of broad bean, corn or radish.

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