Incorporation of 5-Bromo-2-deoxyuridine into the Chloroplast DNA of *Anemia phyllitidis*

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5-Bromo-2-deoxyuridine (BUdR) is incorporated into chloroplast DNA of *Anemia phyllitidis*. A possible connection between previously published effects of BUdR and on chloroplast ultrastructure and transitions caused by the base analogue in the DNA is referred to.

Introduction

As pointed out previously [1], 5-bromo-2-deoxyuridine (BUdR) causes significant changes in the ultrastructure of chloroplasts in the prothallia of *Anemia phyllitidis*. Since natural mutants show comparable modifications in chloroplast structure there is reason to assume an analogous effect at the level of processing of the genetic information. Since chloroplasts are partially autonomous with respect to the coding of their constituents they possess targets for mutagenic factors.

BUdR is an analogue of deoxythymidine and is reported to substitute for it partially [2–6] or totally [2] in the DNA. Its role in the developmental biology has been pointed out, but details about the way in which its effects are brougt about remain still unclear [7].

In the present work the incorporation of BUdR in the chloroplast (cp)-DNA of *Anemia phyllitidis* is investigated.

Material and Methods

Chloroplast of the prothallia of *Anemia phyllitidis* were used. General growth conditions are described in the preceding paper [1]. Essentials about the incubation are pointed out in the legends of the respective figures.

Chloroplast isolation: Prothallia were washed thoroughly with a jet of distilled water. They were homogenised gently in a mortar using the buffer of Jensen and Bassham [8]. Centrifugation steps at $200 \times g$ for 150 s (sediment discarded), at $1500 \times g$ for 7 min and at $1000 \times g$ for 10 min (with the same buffer; supernatant discarded) sufficed to eliminate all recognizable nuclear and mitochondrial residues as checked by staining with acetocarmine respective by electron microscopy.

The nucleic acids were extracted by the diethylpyrocarbonate method described by Solymosy *et al.* [9]. Analytical investigations were done both of the total chloroplast nucleic acids and the cp-DNA isolated enzymatically (RNAase – Merck; Proteinase K – Boehringer). The results of both approaches were identical with respect to the characterization of the cp-DNA.

Isopycnic centrifugation in CsCl gradients was carried out in the IEC/B 60 ultracentrifuge with swinging bucket rotor. Gradients of average density 1.70 g cm^{-3} and those of average density 1.55 g cm^{-3} with ethidium bromide [10, 11] were used.

After the cleavage with DNAase and phosphodiesterase (Boehringer) thin-layer chromatography on polyethyleneimino cellulose (Macherey and Nagel, Düren) in different LiCl solutions served for identification of the nucleotides [12].

Results and Discussion

Buoyant density centrifugation of cp-DNA isolated from untreated *Anemia* gametophytes shows a single peak with a density of 1.704 g cm^{-3}. Since Koop [5] found a density of 1.708 g cm^{-3} for the total DNA of this material, a separation of both components by the drop collecting method is difficult. With respect to this fact his data cannot clearly distinguish the nuclear and chloroplastic components.

After 7 days of incubation of the plants with radioactive BUdR (2 μCi/μ mol) there appears in the density gradient separation a shoulder at about 1.749 g cm^{-3}, which, together with the increasing specific labelling from the upper to the lower parts

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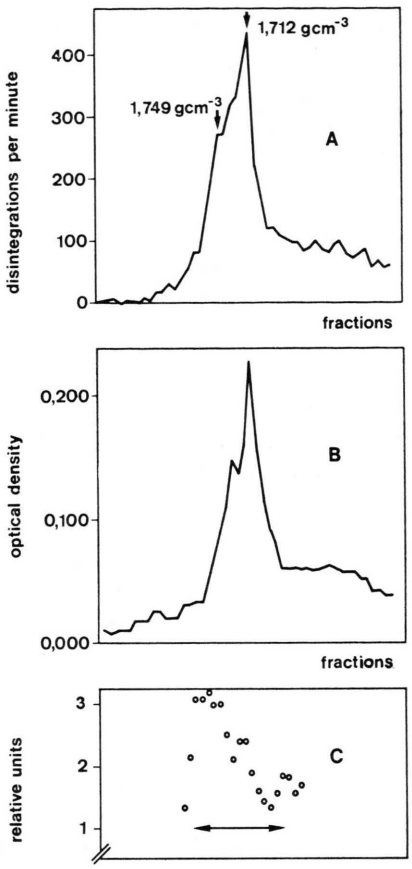

Fig. 1. Density gradient centrifugation of the cp-DNA of 28 days old *Anemia phyllitidis* prothallia after 7 days incubation with [^{14}C]BUdR (10^{-4} M, 2 μCi/μmol). CsCl solution of average density 1.700 g cm^{-3} were spun for 60 hours at 33 000 rpm in IEC B-60 ultracentrifuge, swinging buckett rotor (498). A, radioactivity profile; B, extinction profile; C, specific labelling rate in relative units.

of the gradient, indicate the incorporation of BUdR into the cp-DNA (Fig.1). Also a slight shift of the main peak to 1.712 g cm^{-3} is significant (Fig. 1). The peak includes however also the cold tracer DNA (1 : 1).

Hydrolysis of cp-DNA, labelled with [^{14}C]BUdR gave raise to three labelled spots after thin-layer separation. These spots correspond with bromodeoxyuridine monophosphate, bromodeoxyuridine and bromouracil. All other deoxynucleotides prooved to be free of labelling.

Longer treatments were made to reach a significant density labelling. Since CsCl centrifugation in presence of 500 μg/ml ethidium bromide [10, 11] allows an easy evaluation of density patterns even in unlabelled nucleic acids, this method was used to cross-examine the labelling experiments. For this purpose photographs of the tubes were made under UV-light (365 nm) and densitometric traces of the photographs were compared. The superposition of results after increasing times of BUdR treatment is shown in Fig. 2 (see also Fig. 4). A significant shift towards the denser bands can be observed. The middle band (arrow) appears after recentrifugation in CsCl without ethidium (average density of gradient 1.70 g cm^{-3}) at 1.752 g cm^{-3} (Fig. 3). This is the density of half (one strand) – saturated DNA as calculated according to Luk and Bick [13]. This value corresponds with those of the shoulder appearing in the radioactivity profile shown in Fig. 1. It indicates 100% substitution of BU for T in cp-DNA of *Anemia*. This finding differs with the data about the total DNA of *Anemia phyllitidis* presented by Koop [5]. However, significant longer treatments in our case must be taken into account.

Seyer and Lescure [14] found a higher BU/T substitution rate in the cp-DNA of tobacco cell cultures than in the total DNA preparation. However, they argue that nuclear contamination could cause some misinterpretation.

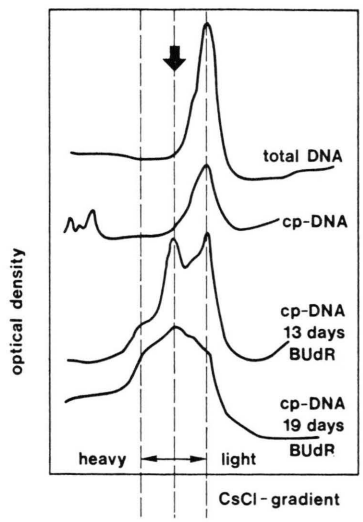

Fig. 2. Densitometric traces of different nucleic acid preparations centrifuged in CsCl (average density 1.55 g cm^{-1}) with ethidium bromide (0.5 mg/ml). (60 hours at 33 000 rpm in IEC B-60 ultracentrifuge swinging buckett rotor) 10^{-4} M BUdR was used from the 14th day of the prothallia culture and was applied by daily change of the incubation solution. Duration of the treatment is enclosed to every individual scan. Arrow signifizes the peak to be recentrifuged (see Fig. 3).

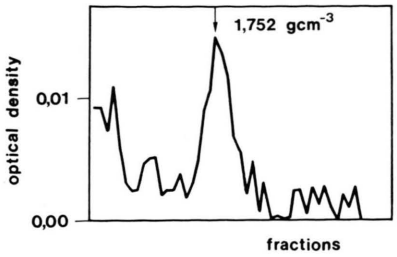

Fig. 3. The band signified in the Fig. 2 was recentrifugated in CsCl-gradient average density 1.700 g cm^{-3} to determine the standard density without ethidium bromide.

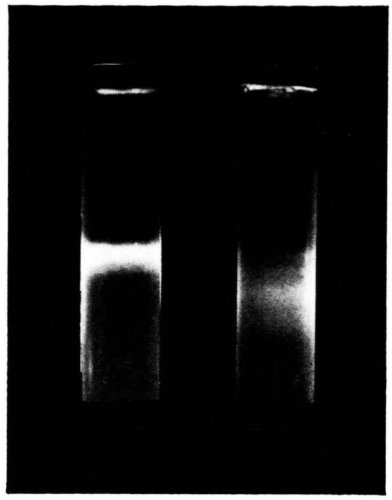

Fig. 4. Comparison of chloroplast DNAs from control and treated plants (13 days preculture followed by 13 days 10^{-4} M BUdR treatment) after centrifugation in CsCl (average density 1.55 g cm^{-3} + ethidium) 60 hours at 33 000 rpm in IEC B-60 ultracentrifuge, swinging buckett rotor (498). Photographs of fluorescence in UV.

Our results demonstrate that BUdR is incorporated into chloroplast DNA in prothallia of *Anemia phyllitidis*. This suggests that incorporation of BUdR into the cp-DNA may mediate the previously published [1] effects of the analogue on the structure of the chloroplasts.

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