MORE EVIDENCE FOR BACTERIA-LIKE PROTEIN SYNTHESIZING APPARATUS IN CHLOROPLASTS AND MITOCHONDRIA

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From various findings it is suggested that chloroplasts and mitochondria possess an autonomous apparatus for protein synthesis [1,2]. This is confirmed by experiments described in this paper. It is shown that in a DNA-dependent cell-free protein synthesizing system from *E. coli* [3] where bacterial or bacteriophage DNA's but – with one exception discussed later – not nuclear DNA's from eucaryotic organisms stimulate amino acid incorporation, the DNA's of mitochondria and chloroplasts possess a significant activity in stimulating protein synthesis. Experimental results, which will not be described in this paper in detail show that the difference of activities of DNA's in this system is based on a messenger-ribosome interaction, i.e. ribosomes from *E. coli* cannot work with mRNA transcribed from nuclear DNA's [4]. On this basis the protein synthesizing machineries of different organisms and organells can be divided into a "bacterialike" and a "non bacterial" group.

Differences between the protein synthesizing apparatus of chloroplasts and mitochondria on the one hand and that of the corresponding cytoplasm on the other hand [5] have been found in the size of the ribosomes (about 70S and 80S respectively) [2,6,7,8], in a different sensitivity against certain antibiotics [9–12] and in the initiation mechanism of translation [13,14]. In these characteristics chloroplasts and mitochondria are very similar to bacteria. Since, however, evidence has been presented that morphological and functional characteristics of protein synthesizing machineries do not always agree [4,15], it appeared necessary to classify the chloroplast and mitochondrial protein synthesis systems also functionally.

As shown in table 1, DNA from mitochondria (rat liver) as well as from chloroplasts (spinacia oleracea) stimulate protein synthesis in the *E. coli* system [16]. Nuclear DNA from rat liver gives no significant stimulation of protein synthesis. Surprisingly, DNA from spinach nuclei definitely shows a stimulation effect, which at least in part, might be due to the presence of plastid genetic information in nuclear DNA as indicated by hybridization experiments [17].

The evidence presented here together with the mentioned previous findings demonstrate the existence of a bacterialike protein synthesizing apparatus in chloroplasts and mitochondria.

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Table 1
Stimulation of leucine incorporation by saturating amounts of different DNA's.

	DNA source				
	Chloroplast	Spinach nuclei	Mitochondria	Rat liver nuclei	T_4
n moles ^{14}C-leu incorporated	5.2–6.8	2.4–3	3– 4	<0.5	20
% of T_4-DNA stimulation	26–34	12–15	15–20	<3	100

The assays for protein synthesis (incubation time 40 min) were performed as described previously [3]. Background incorporation was between 0.2 and 0.5 n moles ^{14}C-leucine. The incorporation of ^{14}C-leucine is DNA-dependent as shown by inhibition with actinomycine D and DNase or by omitting DNA. It was completely depressed by chloramphenicol. DNA from bacteriophage T_4 which is a very active template in this system, was taken as a standard. T_4-DNA was prepared as described previously [3]. DNA's from chloroplasts and spinach nuclei were prepared according to Whitfeld and Spencer [18]. Nuclear spinach DNA was free from chloroplast DNA as estimated by the methylcytosine content [18] and DNA saturation curves. Rat liver nuclei were prepared according to reference [19] and mitochondria according to Schneider [20]. Further purification of the mitochondria was achieved by centrifugation through 54% sucrose onto a layer of 56% sucrose. DNA's from these sources were isolated by the phenol-SDS-method [21]. All DNA's were further purified by preparative CsCl-density gradient centrifugation and tested in analytical CsCl-density gradient runs.

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