IN VIVO DIFFERENTIATION OF EUGLENA CYTOPLASMIC AND CHLOROPLAST PROTEIN SYNTHESIS WITH CHLORAMPHENICOL AND DL-ETHIONINE

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Antibiotics such as chloramphenicol, tetracycline, erythromycin, etc. more effectively inhibit in vivo mitochondrial than cytoplasmic protein synthesis in yeast (Clark-Walker & Linnane, 1966). We here confirm their observation of a difference between in vivo sensitivities of cytoplasmic and organelle protein synthesis to chloramphenicol. We extend this difference to protein synthesis in the Euglena chloroplast. Chloramphenicol and DL-ethionine inhibited in vivo protein synthesis more in chloroplasts than in cytoplasm.

RESULTS

Euglena gracilis strain Z grown heterotrophically in the dark synthesizes no chlorophyll and little carotenoid. Such cells, washed 2X in distilled water and suspended in a stasis medium in the light, synthesize large quantities of the proteins of the photosynthetic apparatus (Brawerman & Konigsberg, 1960, Gross & Wolken, 1960, App & Jagendorf, 1963); chlorophyll and carotenoid also increase. Chloramphenicol and ethionine inhibited synthesis of these pigments, presumably by inhibiting the proteins needed for their synthesis, at concentrations not inhibiting cytoplasmic protein synthesis (expressed as multiplication or cell O. D.), (Tables I and II). Pigment synthesis was inhibited in multiplying and resting cell suspensions.

TABLE I

EFFECT OF CHLORAMPHENICOL AND DL-ETHIONINE ON PIGMENT SYNTHESIS IN A RESTING *EUGLENA* SUSPENSION

		Incubation Time (hours)		
		0	24	48
Compound	Conc. (mg%)	% Transmission at 665 mμ *		
None		99	98	69
Chloramphenicol	1	98	99	75
	10	98	99	96
	30	98	100	97
	60	98	99	97
	100	98	100	99
None		98	94	74
DL-Ethionine	0.03	98	95	78
	0.1	98	96	92
	0.2	98	97	94
	0.3	98	98	96

Dark-grown cells were suspended in tris-maleate buffer, pH 7.0, and exposed to 150 foot-candles of fluorescent light on a reciprocal shaker.
*Bausch & Lomb Spectronic 20 was used to determine transmission of methanol extract of cells.

DISCUSSION

Chloramphenicol inhibited *in vitro* protein synthesis in *Euglena* chloroplasts and chloroplast ribosomes at concentrations not affecting protein synthesis in *Euglena* cytoplasmic ribosomes (Eisenstadt & Brawerman, 1964). This difference in protein synthesis is here extended to *in vivo* protein synthesis in *Euglena*. Organelle protein synthesis in *Euglena* as in yeast (Clark-Walker & Linnane, 1966), is different than

TABLE II

EFFECT OF CHLORAMPHENICOL AND DL-ETHIONINE ON *EUGLENA* MULTIPLICATION (CYTOPLASMIC PROTEIN SYNTHESIS) AND PIGMENT SYNTHESIS*

Compound	Conc.(mg%)	Cell O. D.	% Transmission at 665 mμ **
None		1.50	10
Chloramphenicol	1	1.51	8
"	10	1.61	11
"	30	1.41	27
"	60	1.21	29
"	100	1.51	91
None		1.76	41
DL-Ethionine	0.03	1.76	47
	0.1	1.76	53
	0.2	1.75	64
	0.3	1.73	77

*Procedures for multiplication described elsewhere (Aaronson & Bensky, 1962)
**Spectronic 20 used to determine transmission of a 10ml methanol extract of cells whose multiplication is indicated by O. D. (optical density). Carotenoids gave similar results.

cytoplasmic protein synthesis in its sensitivity to chloramphenicol; organelle protein synthesis resembles bacterial protein synthesis. *Euglena* chloroplasts (Brawerman & Eisenstadt, 1964, Edelman *et al.*, 1964, Ray & Hanawalt, 1964) and yeast mitochondria (Schatz *et al.*, 1964, Tewari *et al.*, 1965) also contain DNA and DNA-polymerase has been found in yeast mitochondria (Wintersberger, 1966). It becomes clear that organelles such as mitochondria and chloroplasts have much of the biochemical equipment associated with independent cells and microorganisms, i.e. an ATP generating system, information storage, and macromolecule

synthesis. Thus, their intracellular multiplication becomes more reasonable and their origin from free-living microorganisms becomes less improbable.

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REFERENCES

Aaronson, S. & Bensky, B. (1962) J. Gen. Microbiol. 27, 75.
App, A. A. & Jagendorf, A. T. (1963) J. Protozool. 10, 340.
Brawerman, G. & Eisenstadt, J. M. (1964) Biochim. Biophys. Acta 91, 477.
Brawerman, G. & Konigsberg, N. (1960) Biochim. Biophys. Acta 43, 374.
Clark-Walker, G. D. & Linnane, A. W. (1966) Biochem. Biophys. Res. Commun., 25, 8.
Edelman, M., Cowan, C. A., Epstein, H. T. & Schiff, J. A. (1964) Proc. Natl. Acad. Sci. U.S. 52, 1214.
Eisenstadt, J. M. & Brawerman, G. (1964) J. Mol. Biol. 10, 392.
Gross, J. A. & Wolken, J. J. (1960) Science 132, 357.
Ray, D. S. & Hanawalt, P. C. (1964) J. Mol. Biol. 9, 812.
Schatz, G., Haslbrunner, E. & Tuppy, H. (1964) Biochem. Biophys. Res. Commun. 15, 127.
Tewari, K. K., Jayaraman, J. & Mahler, H. R. (1965) Biochem, Biophys. Res. Commun. 21, 141.
Wintersberger, E. (1966) Biochem. Biophys. Res. Comm. 25, 1.