Protein synthesis by developing plastids isolated from *Euglena gracilis*

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1. INTRODUCTION

Euglena gracilis has been widely used as a model for the study of light-induced plastid development [1]. When cells that have been grown in the dark are exposed to light, the proplastids differentiate rapidly into mature, functional chloroplasts. Although the concept that plastid and nuclear genomes share control of chloroplast development has become widely accepted, there is little direct information on the contributions of the plastid itself to this process [2–7]. The development of a method for the isolation from *Euglena* of chloroplasts capable of rapid protein synthesis [8,9] encouraged us to investigate the expression of plastid genes by examining the products of protein synthesis in organello over the course of light-induced development. In addition to finding increases in the synthesis of certain polypeptides (as observed in [3,5,10]) we find a number of high M_r polypeptides which are synthesized transiently during early stages of development.

2. MATERIALS AND METHODS

2.1. *Growth of the organism*

Euglena gracilis (Klebs) strain z was grown in the dark at 22°C on a glucose-containing medium that was limiting in cyanocobalamin [8] and transferred at different times to light of 2×10^{15} quanta $\cdot s^{-1} \cdot cm^{-2}$.

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2.2. *Isolation of plastids*

Mature chloroplasts were isolated from spheroplasts of *Euglena* by mild shear in a Waring Blendor followed by sedimentation into density gradients of silica sol, as in [8,9]. For cells exposed to <6 h light, centrifugation in the density gradient step was increased to 10 000 rev./min for 40 min. The purified plastids from 1–8 liters culture were washed and resuspended in a 5 ml sorbitol–tricine (0.33 M sorbitol, 50 mM tricine (pH 8.4)). All solutions and glassware were sterilized to minimize bacterial contamination.

2.3. *Protein synthesis*

The plastids were incubated at 20°C with 50 nM [^{35}S]Met (New England Nuclear, Boston MA) in 2×10^{16} quanta $\cdot s^{-1} \cdot cm^{-2}$ red light for 30 min. Samples of 25 μl were taken at 10 min intervals and blotted on Whatman filter discs. Discs were prepared for scintillation counting as in [11]. For experiments measuring protein synthesis using exogenous ATP, an ATP-regenerating system [12] was used containing ATP (20 mM), creatine phosphate (50 mM), and creatine phosphokinase (1 mg/ml). Following incubation, the plastids were washed in sorbitol–tricine, suspended in solution A of [13], and stored in liquid nitrogen in the presence of ~1 mM phenylmethylsulfonylfluoride.

2.4. *Electrophoresis and fluorography*

Solution B of [13] containing SDS was added to the samples and aliquots containing ~100 μg protein were applied to SDS–polyacrylamide gradient gels (7.5–15% polyacrylamide). Electrophoresis was allowed to continue overnight. Gels were stained with Coomassie brilliant blue and treated

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with En^{3}Hance (New England Nuclear, Boston MA). The dried gels were analyzed by fluorography [14].

3. RESULTS

3.1. *Protein synthesis*

Protein synthesis was measured by the incorporation of [^{35}S]Met into normal, green chloroplasts and plastids from cells exposed to light for 0, 1, 3, 6, 9, 12 and 24 h before isolation (fig.1).

The rates of incorporation into developing plastids increased progressively with the time of development. Proplastids incorporated [^{35}S]Met at the same very low rate whether or not exogenous ATP was supplied. At later stages of development the addition of ATP in the presence of light was not only unnecessary, but actually decreased the incorporation of [^{35}S]Met in 30 min by ~2-fold (fig.2).

3.2. *Polypeptide patterns of developing plastids*

Electrophoresis of the plastid polypeptides resulted in the stainable gel patterns on the left side of fig.3. Although the amounts of several polypeptides change during development, the patterns remain for the most part unchanged. The low concentration of polypeptides in the proplastid lane (0) is due to a systematic overestimation of the protein content of proplastids. We have observed repeatedly that samples of normal chloroplasts and proplastids that contained equal amounts of proteins when assayed with Coomassie brilliant blue G-250 showed disparate concentrations of polypeptides when stained in the gel with Coomassie brilliant blue R-250.

The translation products of the developing plastids are seen in the fluorograph on the right side of fig.3 and in fig.4. Nine plastid translation products are unique to plastids from cells exposed to 1 h and 3 h light. Most of these products are $> M_r$ 70 000. Six of them migrate with weakly stained polypeptides. Even when the fluorographs are greatly overexposed, we are unable to detect any of these novel polypeptides in proplastids or in plastids from later stages of development (fig.4). These unusual translation products are not observed in every preparation; the pattern of their

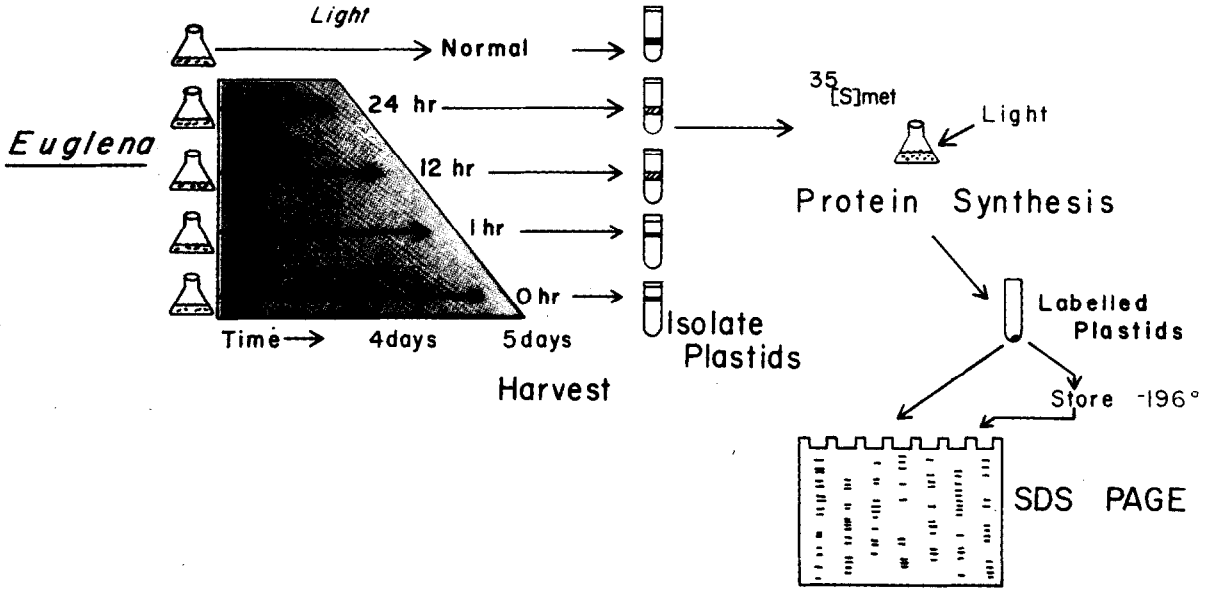

Fig.1. Strategy of experiments. Dark-grown *Euglena* were transferred to the light at various intervals. The plastids were isolated in density gradients of silica and incubated with [^{35}S]Met. The plastids were then collected and the polypeptides of the plastids analyzed by SDS–PAGE and fluorography.

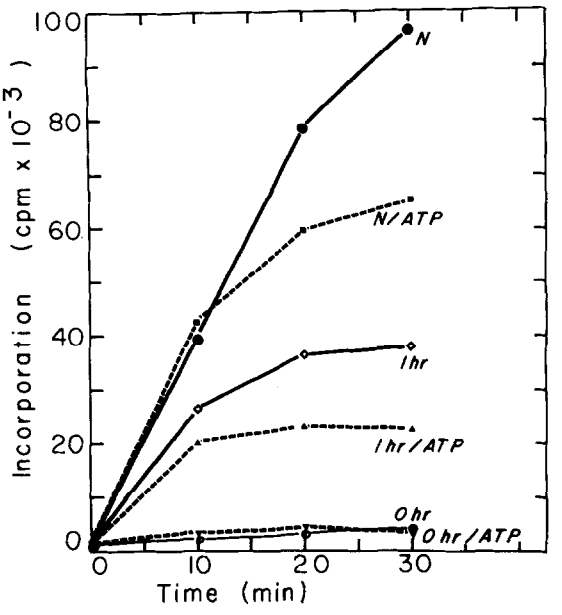

Fig.2. Protein synthesis by normal and immature plastids. Protein synthesis was measured by the incorporation of 50 nM [^{35}S]Met into plastid protein with and without the addition of ATP. Proplastids (○) 0 h; (▾) 0 h/ATP [1] incorporated at the same rate whether or not ATP was present, whereas incorporation by 1 h (◇:▲) and normal (●:■) plastids was decreased by the addition of ATP.

appearance suggests that their occurrence is highly dependent on the precise stage of light-induced development of the cells. Although the absolute rates of protein synthesis by proplastids are very low (fig.2), comparison of the pattern of protein synthesis by proplastids with that of mature chloroplasts shows them to be qualitatively similar (fig.4).

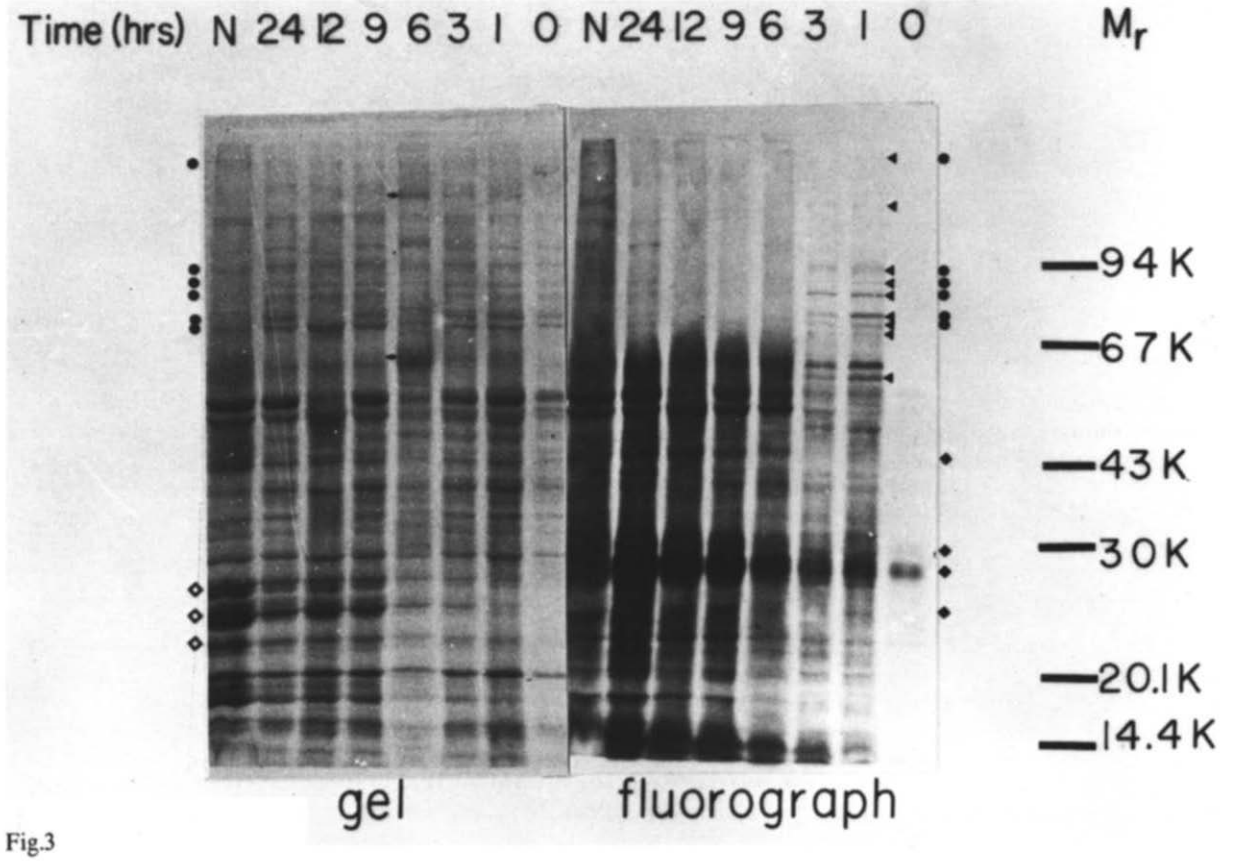

Fig.3

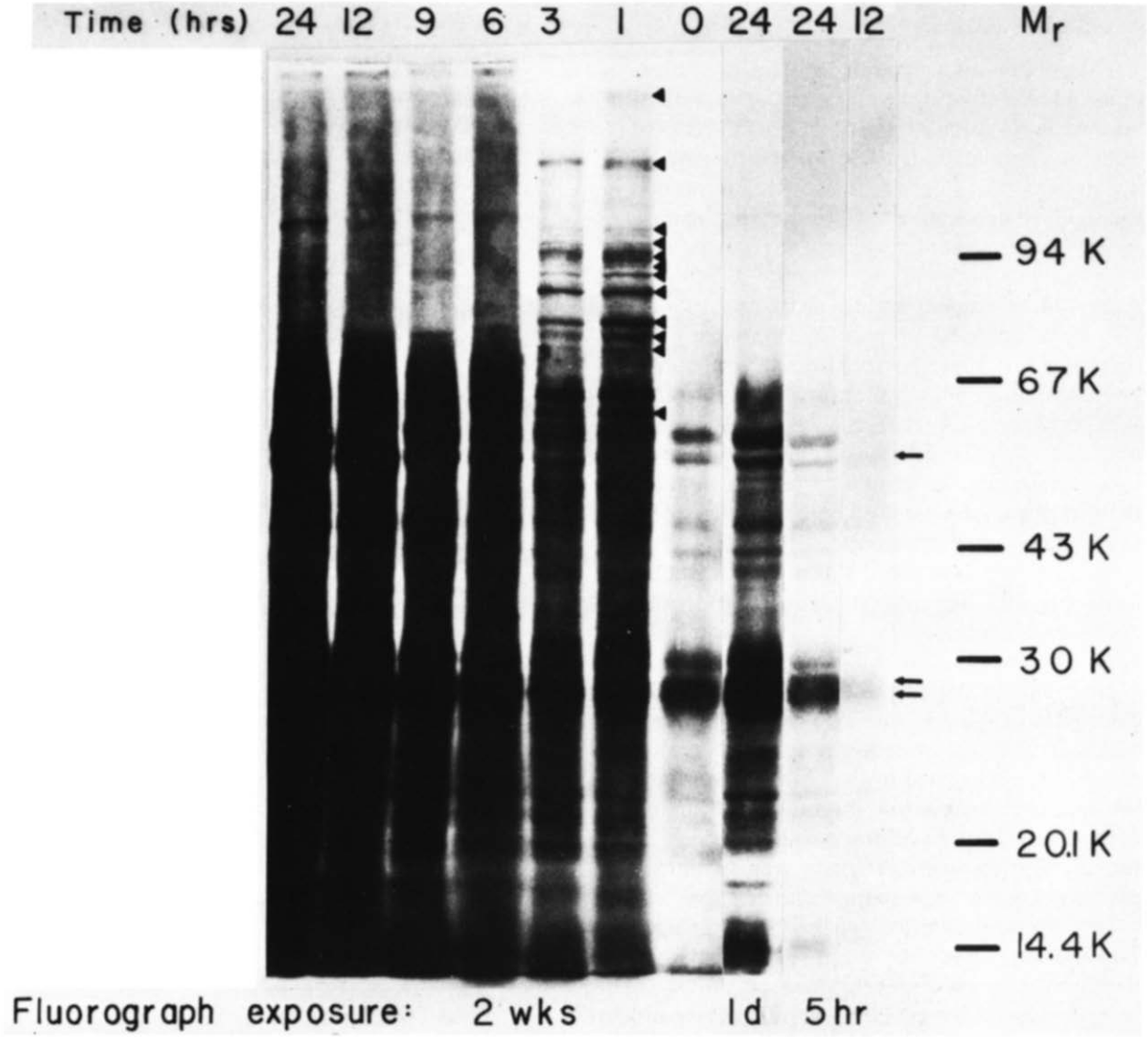

Fig.4. Comparison of short and long exposures of fluorographs of plastid proteins. X-ray films were exposed to the same gel shown in fig.3 for intervals of 5 h, 1 day and 2 weeks. A 2 week exposure of translation products from developing plastids shows additional transient, high M_r translation products (◀) in proteins from 1 and 3 h plastids and that few if any of these are detectable above background in plastids from later stages. Long exposure of proplastid polypeptides (○) revealed a number of translation products not seen in the proplastid lanes of fig.3. The pattern of polypeptides synthesized by proplastids is very similar to that of 24 h plastids (cf. 1 day and 5 h fluorographs), but is quite distinct from those in early development (1 and 3 h plastids).

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Fig.3. SDS-gel electrophoresis and fluorography of total plastid proteins during light-induced development. Plastids labelled with [^{35}S]Met were subjected to electrophoresis in SDS–polyacrylamide gradient gels and fluorographed for 2 days. Left: gel pattern of polypeptides stained with Coomassie blue from proplastids (0) and plastids isolated after 1, 3, 6, 9, 12 and 24 h greening; (N) represents proteins from normal, green chloroplasts. Some polypeptides increase many fold during development (◇). A few appear at 1 h development and disappear after 6 h (→). Right: Fluorography of labelled polypeptides. Transient polypeptides of high M_r (◀) are seen only during the first 3 h development. Certain of these translation products comigrate with stainable polypeptides throughout development (•). The rates of synthesis of several other polypeptides (◆) increase dramatically during development.

4. DISCUSSION

The models that emerge from most studies of light-induced plastid development include the differential expression of plastid genes [2–7,15,16]. A number of polypeptides, notably the triazine-binding protein [15,16], are formed after an interval in the light, whereas others are formed in the dark as well. We see a similar pattern here.

Transient, high M_r polypeptides, appearing early in development and then disappearing, have also been reported in plastids of maize [3] and cucumber [5]. These polypeptides were reported to be polypeptides of cytoplasmic origin, although R. Walden (personal communication) also found traces of them along the translation products of immature cucumber plastids. We find in *Euglena* that incorporation of [^{35}S]Met into these novel polypeptides may represent a significant fraction of protein synthesis during the earliest stages of development. They range upward from M_r 60 000 and several stain weakly with Coomassie brilliant blue.

High-M_r translation products were found [17] in chloroplasts isolated from fully greened *Euglena* in which cytoplasmic protein synthesis had been suppressed. We suggested in that communication that the aberrant translation might be precursors of normal proteins whose processing had been interrupted. The transient products seen in immature plastids may also represent precursors.

In [6,7] certain portions of the plastid genome in *Euglena* were shown to be transcribed only during the first few hours of development. If the transient polypeptides observed here should correspond to these transcripts, they would represent proteins truly unique to early development and not to the accumulation of processed intermediates. A full understanding of plastid development requires that we distinguish between these alternatives.

ACKNOWLEDGEMENTS

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