SYNTHESIS OF PROTEIN DURING THE NUCLEAR DIVISION CYCLE IN *PHYSARUM POLYCEPHALUM*

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1. Introduction

In a plasmodium of the Myxomycete *Physarum polycephalum* the nuclei divide synchronously, and the organism is therefore ideal for studying biochemical processes in relation to the mitotic cycle. However, its form of growth, as a single, large cell, creates difficulties in taking samples to measure growth or the synthesis of cellular components. Thus, experiments with plasmodia have usually involved taking portions of a fixed area from a single culture (for, say, pulse-labelling with a radioactive precursor) or sacrificing individual plasmodia from a replicate series as a function of time during the mitotic cycle. Such procedures cannot give very good precision in defining patterns of synthesis of macromolecules.

We have developed an isotope-dilution method [1] which can be used to study the synthesis of any macromolecule that is metabolically stable and which does not involve the difficulties of interpretation of pulse-labelling experiments. Another important advantage of the method is that it does not require all the samples taken in one experiment to be identical in size. The procedure has been used to derive the patterns of synthesis of DNA and rRNA [1] and tRNA [2] during the nuclear division cycle.

Determination of the accumulation curve for total protein by direct application of the isotope-dilution method, prelabelling the inoculum with a radioactive amino acid and then measuring the decrease in the specific activity of plasmodial protein, would not be acceptable because many proteins probably have half-lives that are significantly short in relation to the growth rate of a plasmodium [3]. However, the isotope-dilution method can be applied indirectly by prelabelling the metabolically stable nucleic acids and then, in samples from a surface plasmodium, determining both the specific activity of the nucleic acid (S) and the amounts of nucleic acid (N) and protein (P). Since S is proportional to N^{-1} [1], the relative accumulation curve for protein is given by $S \cdot N^{-1} \cdot P$. This paper describes the results obtained for the synthesis of protein during the mitotic cycle using this method.

2. Materials and methods

Stock cultures of microplasmodia of strain M_3C VIII were maintained at 26°C in medium containing tryptone (Difco) and yeast extract [4]. Large surface plasmodia were grown as described previously [1,2]. Microplasmodia were labelled with [2-^{14}C]uridine (10 μM; 0.6 μCi ml^{-1}) rather than [5,6-^{3}H]uridine that was used in earlier work [2]; the procedure for labelling was otherwise unchanged.

Segments (approx. 3 cm^2) of plasmodium were homogenised in 5% (w/v) trichloroacetic acid in H_2O : acetone (1:1) and the total content of nucleic acid and protein determined by standard methods [5]. The radioactivity of the nucleic acid hydrolysate in 0.5 M $HClO_4$ was determined in a liquid scintillation spectrometer using an aqueous scintillant.

The data for the synthesis of total nucleic acid and protein between two successive mitotic divisions were analyzed by fitting to a polynomial of degree n by a least squares procedure using a computer programme.

3. Results and discussion

We have previously used the isotope-dilution method to follow the synthesis of individual classes of nucleic acid (DNA and rRNA [1]; tRNA and rRNA [2]). Figure 1, in contrast, shows the pattern of accumulation of total nucleic acid between post-fusion mitoses II and III in a surface plasmodium. The increasing rate of synthesis towards the end of the mitotic cycle (G2 phase) is characteristic of the synthesis of rRNA [1], which constitutes a major fraction of the nucleic acid [5]. The occurrence of S phase immediately after mitosis [1,6] produces a relative straightening of the line for total nucleic acid synthesis compared to that for rRNA alone [1].

The accumulation curve for protein in the same experiment as that shown in fig.1 is given in fig.2. Because of the greater number of measurements required to obtain the protein values, the errors are larger than those evident in fig.1. However, fig.2 is a representative result and it demonstrates that the synthesis of protein between successive nuclear divisions is non-linear in form, the best fit being to a third order polynomial. There is of course no a priori reason why the pattern of synthesis of the total protein complement of the cell should be mathematically simple in form, given the complexity of the population of molecules involved. Nevertheless, it represents an important parameter in terms of understanding the economy of the cell during the mitotic cycle. Apart from the fact that the synthesis of protein appears to be continuous throughout the cycle, the other interesting feature is the increase in the rate of protein synthesis in G2 phase, which is also the part of the mitotic cycle associated with the maximum rate of ribosome production [1].

Pulse labelling of plasmodia with [^{3}H]lysine during the mitotic cycle has shown a biphasic pattern of incorporation into protein [7], with minima at the time of nuclear division and approximately halfway through the cycle. However, the radioactivity incorporated into protein was not corrected for possible variation in the specific activity attained by free lysine in the amino acid pool in the different samples and so direct comparison with our results is not

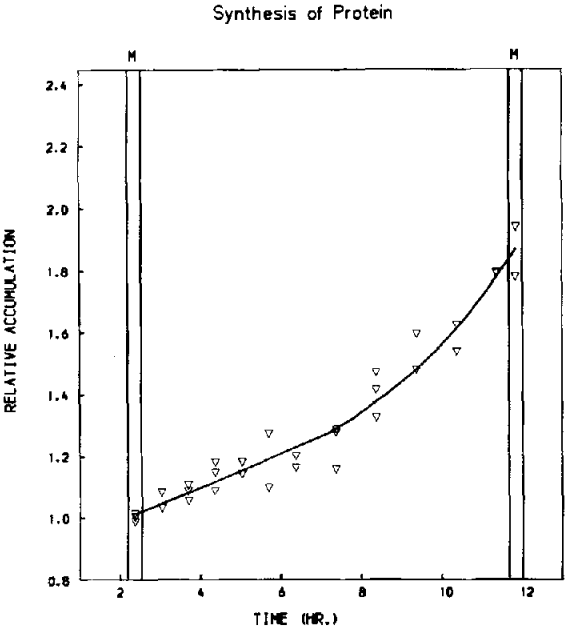

Fig.2. Synthesis of protein between post-fusion mitoses II and III, normalised to a value of 1.0 at MII, in the same plasmodium used for the experiment shown in fig.1.

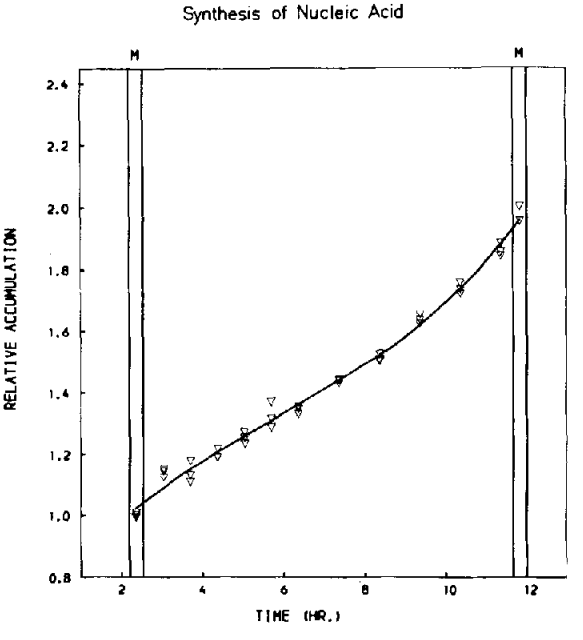

Fig.1. Synthesis of total nucleic acid between post-fusion mitoses II and III, normalised to a value of 1.0 at MII.

possible. Examination of the size distribution of polyribosomes has not revealed any marked variation during the cycle [8], even at mitosis, in contrast to the results obtained with mammalian cells [9,10].

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