Magnesium and control of mitochondrial morphology in fission yeast

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The importance of magnesium in maintaining ultrastructural integrity and respiratory activity of mammalian mitochondria has been known for many years (e.g. Balscheffsky, 1957; Dow et al., 1970). However, despite their widespread utility in studies of mitochondrial morphology, very few studies in yeasts have been concerned with the role of Mg²⁺ ions in regulating mitochondrial structure and function. Using the fission yeast, Schizosaccharomyces pombe, we describe here how magnesium availability dramatically influences mitochondrial morphology in vivo.

Culture conditions and Mg²⁺-depiction methods for S. pombe 972h− have been described by Walker & Duffus (1980). Electron microscopy and gas-exchange studies were undertaken as described previously (Walker et al., 1982).

In actively dividing cells of S. pombe grown in the presence of magnesium, only two or three mitochondria per cell are evident in electron micrographs; these organelles describe here systems which allow us to study possible division-specific proteins under conditions which are not obscured by growth. Culture conditions and heat-shock synchronization methods for S. pombe 972h− have been described previously (Walker & Duffus, 1980; Kramhøft & Zeuthen, 1971). Nitrogen-starvation conditions were established by filtering actively dividing cells, followed by rapid resuspension in pre-warmed minimal medium which lacked the NH₄Cl nitrogen source. Protein synthesis was followed by firstly pulse-labelling cells for 10 min with [³⁵S]methionine (2 μCi/ml), which were then homogenized by glass-bead agitation before centrifugation to release cytoplasmic proteins. Electrophoresis was performed in 10% polyacrylamide gels by the method of Laemmli (1970). Gels were stained, dried and processed for autoradiography by standard procedures.

When cells of S. pombe were transferred to nitrogen-free medium, an immediate but transient increase in cell proliferation rate occurred, with a concomitant decrease in cell size. Fantas & Nurse (1977) have reported similar findings in S. pombe after a nutritional shift-down to a poor nitrogen source. In the present work, protein synthesis patterns were followed throughout the period of accelerated cell division, both in random and in synchronous cultures. We have consistently observed that a very few polypeptide bands are effectively 'switched on' at the period coinciding with cell number increase. For example, Fig. 1 highlights two such bands (46 and 27 kDa) which are differentially synthesized in a rapidly dividing synchronous culture of S. pombe.

The rapid-division systems described here effectively eliminate 'growth cycle' protein synthesis and expose 'DNA-division' protein synthesis. However, although present findings point to the existence of division-cycle-specific gene expression in S. pombe and appear to support the cell cycle control model proposed by Kuhn (1982), the possibility cannot be overlooked that the differentially synthesized proteins observed are simply due to an effect of limiting the nitrogen source, rather than cell division. Consequently, we are now aiming to study protein synthesis in analogous systems which do not entail starving for nitrogen. For example, the accelerated division of the 'wee' mutant of S. pombe which occurs on transfer to a restrictive temperature (Nurse, 1975).


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Fig. 1. Protein synthesis in rapidly dividing S. pombe cells pulse-labelled throughout a synchronous division cycle

Cells were treated to five 41°C heat-shocks to induce synchrony, followed by transfer to nitrogen-free medium to induce rapid division. Two major differentially synthesized polypeptide bands at 46 and 27 kDa have been highlighted (●), and the mid-point of synchronous cell number doubling (80 min) is indicated by the arrow.

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Multiple forms of calf prochymosin and chymosin

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Several studies on extracts from calf stomachs of mixed animal origin have demonstrated that the proteolytic enzyme chymosin (EC 3.4.23.4) is not homogeneous. The classical studies of Foltmann (1966) and Foltmann et al. (1977) showed the existence of at least three forms of the enzyme, designated chymosin A, B, and C, and indicated a close similarity in primary structures of the A and B forms. Other workers described the existence of four forms of both chymosin and its zymogen prochymosin by electrophoretic and chromatographic analysis (Asato & Rand, 1971, 1972). Results from recent studies in molecular genetics indicated that chymosins A and B differ in primary structure by only one or two amino acids and are genetic polymorphs representing different alleles of a single structural locus (Moir et al., 1982). An understanding of the molecular and/or genetic origin of chymosin heterogeneity is of primary importance in connection with recent interest in producing commercial chymosin from genetically engineered micro-organisms. The objective of the present study was to characterize further this heterogeneity by a study of the enzyme in individual calf stomachs, where complex mixtures of genetic variants would be avoided.

Prochymosin was extracted from individual calf stomachs essentially by the method of Foltmann (1966), but with the alum precipitation procedure of Rand & Ernstrom (1965). Conversion of prochymosin into chymosin was performed at pH 2.0 and 20°C for 1h. Before chromatography on DEAE-cellulose, solutions of prochymosin and chymosin were dialysed against column buffer. Details of the chromatographic procedure are given in the legend to Fig. 1. Authentic samples of chymosins A and B were obtained by chromatographic fractionation of crystalline chymosin [Sigma (London) Chemical Co.] on DEAE-cellulose as for individual calf chymosin. Electrophoresis was carried out on polyacrylamide slab gels either by a modification of the procedure of Davies & Law (1977), with 6m-urea and an acrylamide concentration of 6%, or in the presence of sodium dodecyl sulphate as described by Laemmli (1970).

Chymosin was resolved into two enzymically active