Protein Kinase Activities in Rat Liver Nuclei: Effects of Age and Partial Hepatectomy

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Selective substrates and inhibitors have been used to measure kinases phosphorylating endogenous proteins in rat liver nuclei during growth and regeneration after partial hepatectomy. Peaks in activity were found at 5, 22, and 29 hours after partial hepatectomy. Administration of α₁ and β adrenergic blockers suggested that the Be²⁺ sensitive and cyclic AMP-dependent protein kinases were interdependently regulated by Ca²⁺ and cyclic AMP.

INTRODUCTION

Cell cycle associated variations in the phosphorylation of nuclear proteins are well established, and in rat liver after partial hepatectomy the prominent peak in phosphorylation at the G₁/S phase transition is linked with an increase in nuclear cyclic AMP-dependent protein kinase (Laks and Jungmann, 1980). This enzyme is selectively inhibited by Walsh's rabbit muscle protein kinase inhibitor (Walsh et al., 1971). Casein kinase II (or two similar isoenzymes) is present in liver cytoplasm and is also rather firmly bound to liver nuclei. The enzyme is selectively inhibited by heparin (Meggio et al., 1982) and can be activated in vitro by polyamines. This may be significant in the increased phosphorylation in S phase and/or mitosis. Be²⁺ inhibits casein kinase I (Cummins et al., 1982). The kinase activity has now been resolved into two components both of which are Be²⁺ sensitive (Kaser, unpublished). Kinases preferentially phosphorylating histone H1 have also been described (Zeilig and...
Langan, 1980; Quirin-Stricker, 1984; Farago et al., 1986). The availability of the specific inhibitors and of GTP as a selective phosphate donor, permitted kinetic analysis of the protein kinase activities found in liver nuclei isolated from normal rats of various ages or at different times after partial hepatectomy.

**MATERIALS AND METHODS**

**Protein Kinase Assay**

Nuclei were prepared by the method of Chauveau et al. (1956). The incubation medium of 200 mM-Tris-HCl, pH 7.4, 2 mM-β mercaptoethanol, 4 mM-MgCl₂, 4 mM-[γ-³²P]ATP, or [γ-³²P]GTP (specific activities 0.1 Ci/mol) was of a final volume of 150 µl. Nuclei equivalent to 100 µg of DNA were used. Preliminary measurements established the concentrations of substrates and inhibitors to be saturating and the reaction to be linear at 30°C for 20 minutes. Assays were run in triplicate, for 10 minutes. Protein phosphorylation was analysed by electrophoresis on 4.5% polyacrylamide stacking gels/10% running gels (Laemmli, 1970) followed by autoradiography.

[γ-³²P]ATP and GTP were obtained from Amersham International and Walsh's rabbit muscle protein kinase inhibitor from Sigma Chemical Co. Ltd.

**RESULTS AND DISCUSSION**

**Protein Kinase Activities in Normal Liver Nuclei: Variation with Age of Animal**

To study quantitative variations in the capacity of nuclear protein kinases to phosphorylate nuclear proteins through the cell cycle, the type of nuclear preparation used is important. Since extracts (0.35 M NaCl) tend to give irreproducible results nuclei were prepared in hyperosmolar sucrose without prior exposure to a low ionic strength medium.

The extent of histone H1 phosphorylation in liver decreases sharply during the last few days of foetal life (Ord and Stocken, 1969) as the mitotic index in the developing liver falls. There was a 50% fall in kinase activity towards endogenous nuclear proteins and histone H1 at birth (Table 1). With casein as substrate no age variation in levels of phosphorylation was detected. When the relative utilization of substrates is considered the results are consistent with a roughly constant complement of protein kinases being present in liver nuclei, with the addition of a specific, growth-associated histone H1 kinase (cf. Quirin-Stricker and Schmitt, 1981) during foetal development.

No qualitative alterations were detected in the patterns of proteins separating on the gels either with nuclei from rats of different age or after partial hepatectomy. Autoradiographic analysis (Fig. 1A,B) confirmed that protamine phosphorylation was enhanced in nuclei from younger rats and showed that this substrate promoted greater