AIM AND SCOPE

Protein kinases, which catalyze the phosphorylation of key proteins of cellular reactions, have in recent years emerged as an important group of enzymes. Modification of cellular proteins by such phosphorylation influences several critically important cellular processes. Major regulatory proteins, such as enzymes and protein synthesis initiation factors, serve as targets for phosphorylation. This, consequently, controls the nature and efficiency of cellular functions.

In the wake of the discovery of this enzyme system, there was a spate of research into their possible role, mechanism of action and overall physiological significance. There followed an avalanche of reports regarding the nature, effect and properties of these enzymes. The primary interest was generated by the fact that the effect of cAMP is mediated via the cAMP dependent protein kinase. Close in its heed, the cyclic nucleotide independent protein kinases were discovered and more recently it has been shown that some protein kinases are also controlled by effectors other than cyclic nucleotides. In higher eukaryotes, these enzymes have been conclusively shown to be involved in hormone mediated response, control of translation, control of gene activity by phosphorylation of chromatin proteins and a number of other processes. In lower eukaryotes, these enzymes have been reported but

their exact role is not clear. We have chosen yeast, a lower eukaryote, as a model system to study these enzymes, because it is a simple unicellular organism occupying an environment similar to prokaryotes, and can be subjected to genetic analysis.

The role of cAMP in lower eukaryotes, which do not respond to hormones, is not clear. In prokaryotes, the effect of cAMP is to alleviate glucose repression of inducible enzymes. However, in eukaryotes, it is not clear whether these two signals are mutually related. In our laboratory, we have characterized an inducible N-acetyl-glucosamine catabolic pathway in yeast. We desired to study the mechanism of catabolic repression in relation to cAMP using this pathway as a model. We were interested in seeing whether in an inducible system of yeast, glucose and cAMP produce effects similar to those in prokaryotes.

An attempt to elucidate the effect of cAMP in any system invariably leads to a study of protein kinases, which may mediate such effect. A complete analysis of the number and types of protein kinases in a system is, therefore, a prerequisite to further studies. Hence, we followed up our initial studies with an analysis of protein kinases and their endogenous substrates in yeast.

A need for purified preparation of enzymes to study their specific role, initiated us on the problem of purifying a protein kinase from yeast. We decided to purify a protein kinase from yeast by affinity chromatography, with casein as the bound ligand. Cyclic nucleotide independent protein kinases, which phosphorylate casein, have been shown in many organisms. However, the exact physiological role of these casein kinases is not clear. Our purified casein kinase would be useful for future investigation to understand the regulatory role of these enzymes. The method of purification employed in this study is also rapid and simple and may be useful for the purification of other types of protein kinases.

As a part of future studies, it would be interesting to find out the role of protein kinases in translation, in yeast. It would be of interest to see whether the mechanism of translational control shown in mammalian and plant systems, is also operative in lower eukaryotes. Characterization of endogenous substrates of protein kinases, would show which proteins are the targets of its action. It would also be useful to see the effect of these enzymes in transcription and gene activity and cell cycle dependence. Isolation of yeast mutants of protein kinase, lacking in these enzymes or having defective enzymes, would be an extremely useful field for further work. These would provide us with

invaluable information about the mechanism of action of protein kinases. Hence, our initial observations are likely to trigger off a great amount of interesting experimentations, which would help to unravel the role of phosphorylation in relation to gene expression in yeast.
