GENERAL DISCUSSION

In view of the great significance of protein phsophorylaition in biochemical processes, a study relating to the enzymes of phosphorylation, i.e. the protein kinases, is greatly relevant, and perhaps essential to the understanding of the mechanism of gene expression in eukaryotes.

Our initial studies involving the effect of cAMP on an inducible system and its relation to glucose repression interestingly revealed that glucose repression and cAMP action were not related in *Candida albicans* and *Saccharomyces cerevisiae*. On the contrary, cAMP appeared to be involved in the control of protein synthesis. Studies in a number of inducible systems, like that of hepatic enzymes, have indirectly shown that cAMP affects the transcription and translation of mRNA in the induced cells and, thereby, affects induction (235, 236). Also the induction of the different enzymes was shown to be distinctly inhibited by cAMP. The basic mechanism of action of cAMP may, in this case, be through the general inhibitory effect on translation, yet the possibility of a specific action on the induction of enzymes cannot be ruled out.

The general involvement of cAMP dependent protein kinases in mediating the effect of cAMP invariably predicts the involvement of these enzymes in cAMP action. The role of cAMP dependent protein kinase in induction was studied with tyrosine amino transferase (TAT). Protein kinases in

normal rat liver (where induction is present) and rat hepatoma cell line (where TAT cannot be induced by cAMP) were studied. In the hapatoma cells, there was a total lack of type I PK and low concentration of cAMP could induce TAT in normal cells indicating that protein kinases may be involved in induction of TAT(237).

The role of cAMP and protein kinases in protein synthesis is not clear. The mechanism of translational control has been worked out in great detail in reticulocyte lysates. The observation, that cAMP at physiological concentration could inhibit ternary complex formation presumably by activating the proHCI, and that preparation of cAMP dependent bovine heart protein kinase (BHK) catalytic subunitcould show a similar effect as cAMP in hemin supplemented lysates, suggested that cAMP dependent protein kinases may be involved in inhibition. However, catalytic subunits from other sources could not cause such inhibition and a heat stable factor in BHK preparation was shown to cause the inhibition observed earlier. The effect of cAMP was highly reproducible while cGMP was inactive. Hence, the mechanism of translational control by cAMP remains to be understood (238). Though we have, here, demonstrated that cAMP inhibits protein synthesis in yeast and yeasts have protein kinases, whether these enzymes mediate the effect of cAMP or not remains an open question.

Recently, translation control in yeast by hemin has been reported (239). The control of catalase synthesis has been investigated in *in vitro* system. In cell free extracts of heme deficient cells, the translation of catalase mRNA was at a lower rate than that in heme containing cells. However, the similarities in the underlying mechanisms of hemin control in yeast and rabbit reticuloccyte lysate (namely, the presence of a protein kinase controlled by hemin) remains to be explored.

The cAMP independent protein kinases have been studied for their regulatory actions. Several important aspects of the regulation are now evident. Casein kinases are emerging as an important class of regulatory enzymes. Phosphorylation of important cellular protein is catalyzed by casein kinases. It was shown recently that the regulatory subunit of cAMP dependent protein kinase was phosophorylated by casein kinase II, *in vitro* , at a site where endogenous phosphate binds. Peptide maps of the *in vivo* and *in vitro* modified R subunit showed that the same site was modified in both conditions (240). It indicates that protein kinases, within a cell, may interact with each other. Calf thymus RNA polymerase II could be phosphorylated by casein kinase I and II. But this phosphorylation did not alter its activity (241). Casein kinases have been shown to phosphorylate the β subunit of eIF2 and two subunits of eIF3 (242). The importance and function of this phosphorylation remains obscure

and its possible effect on translation is not clear, but it is distinct from the phosphorylation of ∝ subunit of eIF2 which causes consequent inhibition of protein synthesis in retic lysate, due to its inability to bind with another essential factor ESP (243). Recently, it has been reported that a barley embryo casein kinase inhibited protein synthesis in reticulocyte lysate (224). This is the first evidence of a direct effect on protein synthesis by casein kinase. The [illegible] casein kinase, which we have purified, can now be used for studying its effect on protein synthesis. Our casein kinase, classified as a casein kinase I on the basis of its insensitivity to heparin, is, however, inhibited by spermine and spermidine which is an interesting observation. The involvement of polyamines in stimulating protein synthesis may indicate that this casein kinase modulated by the polyamines plays a role in protein synthesis. It is likely that the isolation and purification of the casein kinase from yeast will explain its role in regulation of cellular mechanisms in yeast. Further, studies involving the isolation of other protein kinases and their purifications and purification of endogenous substrate may be of great relevance to the understanding of the role of protein phosphorylation in general.