# GENERAL INTRODUCTION

Phosphorylation and dephosphorylation of uniquely situated proteins is now considered an essential step in cell surface and intracellular regulatory mechanisms. Modification of cellular proteins by phosphorylation have been demonstrated to exert immense effect on controlling cellular mechanisms. It appears that the state of phosphorylation of certain regulatory proteins determines the nature and efficiency of cellular processes. Such phosphorylation of intracellular regulatory proteins has been shown to be catalyzed by a set of enzymes called protein kinases. The reaction catalyzed by these enzymes causes a reversible, posttranslational, covalent modification of proteins by virtue of which the regulatory or enzymatic properties of the protein may be specifically altered. A large variety of resultant effects are shown by such reactions due to their widespread involvement in cellular activities. Such variations occur, due to a range of widely different mechanisms by which a phosphorylated protein acts, though the basic mechanism of phosphorylation is the same for all. For this greatly significant role as a controlling factor in cellular activities, these enzymes and their effectors have been studied and characterized in great detail. The key proteins altered by these enzymes have been found to be

enzymes, acidic or basic cytosolic proteins, mitochondrial proteins, membrane proteins, nuclear proteins, protein factors or proteins of several other organelles.

The reaction catalyzed by protein kinases is the transfer of the terminal phosphate group of a nucleotide triphosphate (NTP) to amino acid residues of protein substrates. The amino acid involved in this phosphate transfer has been shown to be serine and/or threonine in most cases and tyrosine in some cases. The basic reaction involved can be represented by the following equation :

Protein + (n) NTP ⟶ Protein~P + (n) NDP (See Fig.1A)

The nucleotide triphosphate in the reaction is most commonly adenosine triphosphate (ATP). But for certain enzymes guanosine triphosphate (GTP) can also serve as a phosphate donor (1,2). Apart from serine and threonine, histidine and lysine have also been shown to accept the phosphate (3). Phosphorylation by protein kinases requires a divalent metal ion, usually magnesium ($Mg^{++}$), and it is believed that the actual nucleotide substrate is ATP-metal complex. The reaction is universally reversible, however, the reverse reaction is catalyzed by phosphoprotein phosphatase (See Fig. 1B). The balance in the modification of the key proteins is determined by the levels and turnover rates of these two antagonistic reactions. Appropriate signals bring about an alteration in the levels of phosphorylated or dephosphorylated regulatory proteins,

presumably by controlling either the forward or the reverse reaction or both. The reaction involving the protein kinase, is more dynamic of the two and has been studied in detail.

On studying these enzymes from various tissues and organisms, they have been classified using certain criteria of their activities (Table I). Among the first instances of identification of protein kinases, was one from liver acetone powder, which was a casein kinase (4). After the discovery of this casein kinase, cyclic adenosine monophosphate (cAMP) dependent protein kinases were shown to exist while studying the effects of this cyclic nucleotide (5). Furthermore, cyclic guanosine monophosphate (cGMP) dependent protein kinases also exist (6). Recently, calcium ion ($Ca^{++}$) dependent protein kinases were found and these require a protein modulator called calmodulin for activation (7). The double stranded ribonucleic acid (ds RNA) dependent protein kinases were identified in cells treated with interferon and erythrocyte lysates (8).

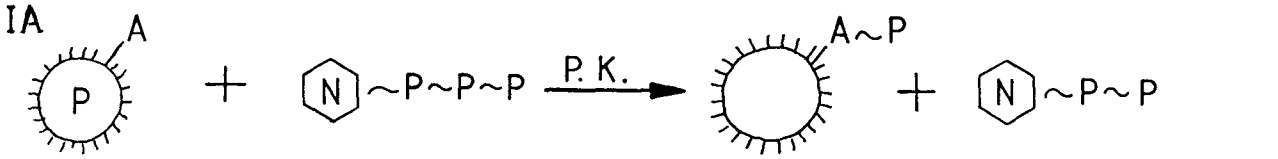
## I. CYCLIC AMP DEPENDENT PROTEIN KINASE

This category of protein kinases are by far the most widely studied and well characterized group of protein kinases. On the basis of their elution from an anion exchange column, these enzymes have been identified to have two isozymes,

FIGURE 1A : SCHEMATIC REPRESENTATION OF PROTEIN PHOSPHORYLATION BY PROTEIN KINASE. 'A' is an amino acid, mostly serine or threonine and sometimes tyrosine. 'A~P' indicates the phosphorylated amino acid. PK denotes protein kinase.

FIGURE 1B : SCHEMATIC REPRESENTATION OF THE ACTION OF PHOSPHOPROTEIN PHOSPHATASE. 'A~P' indicates phosphorylated amino acid in protein. PP denotes phosphoprotein phosphatase.

IA

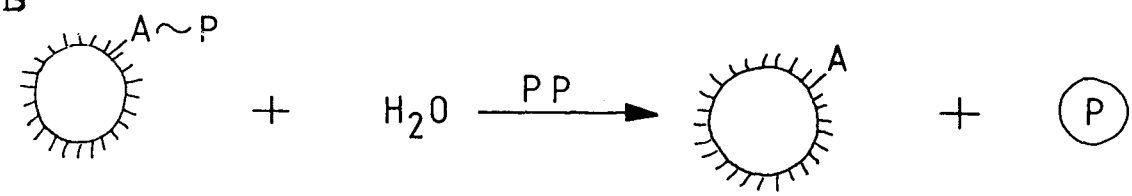



PROTEIN | NUCLEOTIDE TRI PHOSPHATE | PHOSPHORYLATED PROTEIN | NUCLEOTIDE DIPHOSPHATE

PK — Protein kinase

A (amino acid) — Serine
Threonine
Tyrosine

IB




PHOSPHORYLATED PROTEIN. | PROTEIN | INORGANIC PHOSPHATE

PP — Phosphoprotein phosphatase.

A -- Amino acid.

TABLE I

| Category | Designation | Recognised Entities |
|---|---|---|
| 1. | cAMP dependent protein kinase | Type I and Type II |
| 2. | cGMP dependent protein kinase | One known entity |
| 3. | cAMP independent protein kinases: | |
| | a) $Ca^{++}$ dependent protein kinase | Calmodulin activated |
| | | Phospholipid activated |
| | b) Double stranded RNA dependent protein kinase | One known entity |
| | c) Epidermal growth factor stimulated protein kinase | One known entity |
| | d) Hemin controlled repressor | One known entity |
| | e) Casein kinase | Type I and Type II |

called type I and type II. Both types of enzymes have been purified and studied from many different sources. Recently, in neural tissue a subclass of enzyme II has been reported(9). This subclass of protein kinase type II was not detected in any other tissue and this may be indicative of a role of this enzyme, of major importance and specificity in neural tissue.

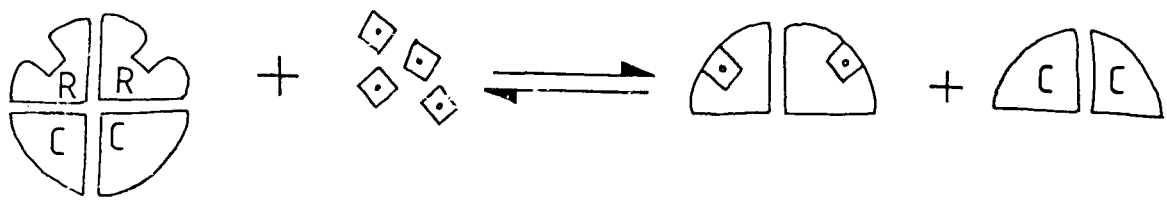
Mechanism of Regulation by cAMP : By studying purified protein kinases it has been shown that these protein kinases are tetramers of two dissimilar subunits. The R subunit represents the regulatory subunit, whereas the C subunit represents the catalytic subunit. In the absence of cAMP these two associate to form a holoenzyme,which shows basal activity. Cyclic AMP caused a stimulation of this activity by binding to the R subunit, with very high affinity, causing a physical dissociation of the two subunits, whereby the active catalytic subunit was released and it exhibited maximal activity. The removal of cAMP caused reassociation of the two subunits to form the holoenzyme. This may be represented as shown in Fig. 2. Cyclic AMP was shown to have a very high affinity for the R subunit which was dependent on protein concentrations (10).

Studies on cAMP binding domain of R subunit have revealed two sites in both types of isozymes. These two are referred to as site 1 and site 2. Experiments on the

FIGURE 2 : SCHEMATIC REPRESENTATION OF THE MECHANISM OF ACTIVATION OF cAMP DEPENDENT PROTEIN KINASE.

'R' represents the regulatory subunit and 'C' represents the catalytic subunit. The inactive enzyme is shown as $R_2C_2$. With the binding of 'R' with cAMP the active catalytic subunit 'C' is released.

II




$R_2C_2$

INACTIVE HOLOENZYME

cAMP

CYCLIC AMP

$R_2(cAMP)_2$

2C

ACTIVE CATALYTIC SUBUNIT

effect of cAMP analogues on cAMP dissociation behaviour suggests that the two sites of one isozyme are quite similar and perhaps homologous to the two sites of the other isozyme. Certain competing nucleotide analogues have been found to inhibit labelled cAMP binding to R subunit by 50%, whereas, much higher concentrations are needed for further inhibition. This indicates that the analogues have a relative preference for either site 1 or site 2 (11). It was also found that cyclic nucleotide analogues with C-8 modification of the purine ring have a relative preference for site 1, whereas those analogues with C-6 modification prefer site 2 (11). That the cAMP binding sites are located in discrete regions of the R subunit, is suggested by the finding that small proteolytic fragments which retain cAMP binding activity can be isolated from both isozymes. The possibility that such cleavage modifies one site such that it does not bind cAMP cannot, however, be ruled out (12,13). A detailed analysis of the function and binding status of the two sites have not yet been worked out, though results suggest that there may be cooperativity between the sites and the binding at one site may stimulate or facilitate the binding at the other site (14).

Mechanism of Regulation by Other Substances : One of the most important modulators of cAMP dependent protein kinases appear to be a heat stable protein inhibitor, which has

been purified from rabbit skeletal muscle (15). This inhibitor binds to the catalytic subunit and inhibits enzymatic activity. A heat stable acidic protein, called the protein kinase modulator, has been purified from lobster tail muscle (16). Another inhibitor was isolated from rat testis (17) and one more from brain called type II inhibitor (18).

One mechanism of modulation of protein kinase was shown to be through ATP-$Mg^{++}$ which appeared to inhibit cAMP binding to kinase and its consequent activation (19). Cyclic AMP dependent protein kinases have been shown to undergo autophosphorylation which may be a possible mechanism of regulation. The C subunit has been shown to incorporate phosphate into the R subunit (20) and it can itself incorporate phosphate upon incubation with ATP and $Mg^{++}$(21). There is evidence that certain hormones like leuteinizing hormone, can stimulate protein kinase purified from bovine corpus leuteum (22).

Modulation of Catalytic Activity : Studies have shown that substrate specificity of cAMP dependent protein kinases may be determined in part, by the requirement of a pair of basic amino acids on the amino terminal side of the phosphorylated serine or threonine (23, 24). It has been postulated that the enzyme recognizes an arginine in the substrate (25). Sulphydryl reagents like N-ethylmaleimide (NEM)

appeared to completely destroy the activity of this enzyme (26), and the enzyme can be partially protected against these reagents by $Mg^{++}$ – ATP. Certain other chemicals like Cibacron Blue F3GA also inhibit the enzyme (27). It has been confirmed that the C subunit has a high specificity for the purine moiety of ATP and therefore the modification of the purine moiety affects the binding. However, analogues modified in the ribose moiety have binding properties similar to ATP (28). Adenine, ADP and AMP can competitively inhibit the enzyme (29).

## II. CYCLIC GMP DEPENDENT PROTEIN KINASE

Convincing evidence regarding the existence of cGMP dependent protein kinases, as a separate entity from the cAMP dependent protein kinases, came from the studies with purified enzymes from beef lung (30). The enzyme was reported to be a dimer consisting of a subunit of molecular weight of about 81,000 dalton. Limited proteolysis of the enzyme leaves a subunit polypeptide which had both cGMP binding activity and catalytic activity. The two activites can be separated on further proteolysis (31). The mechanism of activation can be represented as follows where the cyclic nucleotide-enzyme complex is the active form :

$$E_2 + 2cGMP \rightleftharpoons E_2\ cGMP_2 \qquad \text{(See Fig.3)}$$

The two subunits show cooperativity and have a high apparent affinity for cGMP, which could be non-competitively inhibited by ATP (32). The addition of cold cGMP to cGMP dependent protein kinase, partially saturated with ($^3$H)cGMP, retarded the release of ($^3$H)cGMP. Studies with ($^3$H)cGMP have demonstrated two types of binding sites of this enzyme for cGMP, which have different affinity for cGMP, and different functions (33,34). The ratio between site I and site II was consistently 1:0.5 under various conditions and inclusion of histone increased the binding at both sites.

The cGMP dependent protein kinases have been shown to undergo autophosphorylation and stimulation in the presence of cGMP and cAMP (35). However, there are reports in which autophosphorylation has been shown to be inhibited by cGMP (36) or free regulatory subunit of type I cAMP dependent protein kinase (37). All these properties are very similar to type II cAMP dependent protein kinases. A recent report shows that cAMP stimulated the phosphorylation of cGMP dependent protein kinase and the initial rate of autophosphorylation was slow in the presence of cGMP and rapid in the presence of cAMP (38). On autophosphorylation 4 mol of phosphate was incorporated per mole of enzyme dimer (39).

Modulators of cGMP Dependent Protein Kinase : The cGMP dependent protein kinases also have sites for regulation by regulatory proteins. Calmodulin has been reported to stimulate

FIGURE 3 : SCHEMATIC REPRESENTATION OF cGMP DEPENDENT PROTEIN KINASE ACTIVATION.. 'E' represents the enzyme, which binds cGMP and forms an active enzyme - nucleotide complex.

I


$E_2$
ENZYME

2cGMP
CYCLIC
GMP

$E_2(cGMP)_2$
ACTIVE
ENZYME

this protein kinase (40). Arginine rich histones or poly-arginines have been shown to stimulate this protein kinase activity at lower concentrations, but are inhibitory at higher concentrations. These substances also inhibit cGMP binding (41), suggesting that the catalytic and cGMP binding sites are close. Further studies are needed to elucidate fully, the role of these enzymes in tissues.

## III. CYCLIC NUCLEOTIDE INDEPENDENT PROTEIN KINASES

There are different kinds of protein kinases under this category. These are the calcium dependent protein kinases, the ds RNA dependent protein kinases, the hemin controlled repressor and the casein kinases.

### A. Calcium Dependent Protein Kinases :

The presence and action of calcium in a cell is a general phenomenon and of particular importance is its role in excitable tissues. Calcium has been shown to exert its action via modulators, which act through a regulatory system involving protein kinases. These enzymes are generally called as calcium ion regulated protein kinases. A number of such enzymes have been studied from various tissues. In most cases an intermediate modulator is involved in these reactions. Depending on the modulator, mechanism of action varies and on this basis classification can be made.

Regulation by Calmodulin : One of the modulators of calcium ion dependent protein kinase is a protein called calmodulin (42). Calmodulin is a calcium binding protein and acts in this complex form. Binding of calcium to calmodulin causes a conformational change in the protein, and this active conformer interacts with an inactive or partially active enzyme, which may be represented by the two reactions (i,ii).

$$\text{Calmodulin} + n\text{Ca}^{++} \rightleftharpoons \text{Calmodulin.Ca}_n^{++} \text{ (new conformation)} \quad \ldots\text{(i)}$$

$$\text{Calmodulin. Ca}_n^{++} + \text{E} \rightleftharpoons (\text{Calmodulin.Ca}_n^{++}).\text{E} \quad \ldots\ldots\ldots\text{(ii)}$$

One of the major enzymes modulated by calcium-calmodulin, is myosin light chain kinase. This enzyme activates myosin, by bringing about the phosphorylation of a specific serine residue in it. This forms the biochemical equivalent of contractile activity, which involves the breakdown of $Mg^{++}$-ATP. This enzyme has, therefore, been widely studied in muscle cells (43). The active kinase has been reported to be made up of two nonidentical subunits, the smaller one of which has been identified to be calmodulin itself (43). The $Ca^{++}$ binding subunit,identified to be a modulator protein, is needed for its activity (44). A study of the sequence of events in the activation of the kinase showed, that at a concentration of $10^{-7}$ M of calcium, there was no activation of the myosin kinase, whereas at a concentration of $10^{-6}$M, calcium bound to four binding sites in the calmodulin and under these conditions, calmodulin activated the enzymes(45).

The molecular weight of the enzyme, in smooth muscle, has been demonstrated to be, between 100,000 and 150,000 daltons. Three distinct domains, for the sites of calmodulin binding, phosphoryl transfer and two serine residues which could be phosphorylated by cAMP dependent protein kinase, were identified, and all three could interact with each other. In the presence of EGTA there was no activation, indicating the absolute requirement for calcium (46).

Modulation of $Ca^{++}$-Calmodulin Activated Protein Kinases : The cAMP dependent phosphorylation of the myosin light chain kinase appears to be an important modulation. Studies on this aspect demonstrated that in the presence of bound calmodulin such phosphorylation occurred only on one site, whereas in the absence of bound calmodulin two sites were phosphorylated. Activation by calmodulin was much less efficient if added after phosphorylation (47). Apart from this mechanism, the enzyme has been reported to undergo autophosphorylation (48). Studies with substrates indicate that, a Ser(P)-Asn-Val-Phe primary sequence may be the specific phosphorylation site (49).

Another important class of $Ca^{++}$ activated protein kinase is phosphorylase kinase. This enzyme consists of four types of subunits combined in a tetrameric structure $\alpha_4 \beta_4 \gamma_4 \delta_4$ (50). The $C$ subunit of the enzyme was shown

to have identical amino acid sequence to calmodulin isolated from bovine uterus (51). The $\delta$ and $\gamma$ subunits were tightly bound and did not dissociate during preparation(52). Both the $\alpha$ and $\beta$ subunits could incorporate phosphate upon incubation with $Mg^{++}$-ATP showing autophosphorylation, which could be causing an increase in enzyme activity (53). The mechanism of autophosphorylation is unclear. The subunit exhibited catalytic activity insensitive to $Ca^{++}$, but stimulated by calmodulin (54). Calcium calmodulin regulated protein kinases have been reported to occur in brain (55,56), the physiological properties of which are different from the muscle enzyme. Recently a calmodulin dependent protein kinase was shown to be inactivated by calcium (57). A calcium dependent membrane associated protein kinase which was not pronouncedly sensitive to calmodulin was recently isolated from pea shoots (58). However, from rat pancreatic islet cell membrane, a calcium calmodulin dependent protein kinase and its substrate has been recently isolated. The substrate could be phosphorylated by exogenous addition of $Ca^{++}$ and calmodulin (59).

Regulation by Phospholipids : One class of $Ca^{++}$ activated protein kinase is modulated by phospholipids. These enzymes have been termed as protein kinase C. They have been shown in membranes (60). Protein kinase C was shown to phosphorylate a 40,000 dalton protein and the reaction was absolutely

dependent on $Ca^{++}$, diacylglycerol and phospholipids (61). In rat brain, this enzyme has been studied in different subcellular fractions and these are indistinguishable from each other. Largest fraction was present in crude mitochondrial fraction. The enzyme was shown to be made of a single subunit of molecular weight of 77,000 dalton. The enzyme showed broad substrate specificity and in absence of calcium and diacylglycerol it could phosphorylate protamine. It showed autophosphorylation and consisted of two functionally different hydrophobic and hydrophilic domains (62). In calf thymus, a protein kinase C was shown to phosphorylate histone HI (63). Cardiac troponin or troponin T has been shown to be a substrate for protein kinase C (64).

Phospholipid interacting drugs have been shown to inhibit the protein kinase C. This inhibition is an unique property of the enzyme and the mechanism involved was proposed to be a competitive inhibition with phospholipid but not with calcium (65).

B. Double Stranded RNA Dependent Protein Kinase :

Investigation into the biochemical changes associated with interferon treated or exposed cells led to the discovery of this class of protein kinases. It was detected to be one of the primary enzymatic activity in interferon exposed antiviral cells, on addition of ds RNA (66). ATP was found

to be needed for activation and in the absence of ATP it could show only 12% activity. Enzyme preincubated in the presence of ds RNA and ATP had high activity, whereas pre-incubation in the absence of ds RNA lowered the activity(67). Kinetics of induction of this protein kinase in HeLa cells showed a significant rise between 6-8 hrs on interferon treatment. Actinomycin D could prevent the appearance of the activity, when administered upto 6 hrs after interferon treatment indicating that *de novo* RNA synthesis is needed for the appearance of the enzyme (68). The protein kinase phosphorylated a ribosome associated 72,000 dalton protein and the $\alpha$ subunit of eIF2 (69). A study on the structural requirement of ds RNA for activation of the protein kinase revealed that only ds RNA containing 65-80 nucleotides or longer lengths of poly GC were fully active. dsRNA of poly U; poly A showed similar result and it was indicated that a relatively long stretch of base pair uninterrupted by either a mismatch or discontinuity of one complementary strand is needed (70). Rabbit reticulocytes also had this enzyme and showed that the activation of enzyme took place on the ribosomes and required ATP. High concentrations of poly I, poly C inhibited the activation and its molecular weight was determined to be 120,000 dalton (71). It was shown to be made up of a single subunit protein of 70,000 dalton (72). Recent discovery, from K/Balb cells treated with iododeoxyuridine, of this enzyme has led it to be taken as a biochemical marker of

interferon action (73).

C. Epidermal Growth Factor (EGF) Stimulated Protein Kinase :

This protein kinase was discovered as a receptor for the EGF which is a mitogenic polypeptide hormone. This enzyme has been studied in membrane preparations. A native EGF receptor-kinase complex of 170,000 daltons has been prepared from A 431 tumor cell line. There was a basal intrinsic activity stimulated by EGF. An earlier preparation of 150,000 dalton has been suggested to be a proteolytic product of the 170,00 peptide (74). In rat liver, during regeneration, a dose dependent increase in membrane phosphorylation was observed with EGF in a 170,000 dalton protein (75). This enzyme has been studied by affinity labelling. NEM and heat treatment inhibited the enzyme and the phosphorylation of the protein (76). The phosphorylated product of EGF dependent protein kinase has been identified as a tyrosine residue of both endogenous membrane protein and histone. Only traces, if any, of phosphoserine and phosphothreonine could be detected (77). Recently, the receptor-protein kinase has been purified from liver of normal mice by affinity chromatography. The liver protein kinase showed properties similar to the tumor cell protein kinase. It shows autophosphorylation and phosphorylation of tyrosine residues. These two enzymes from two different sources could also cross react antigenically (78). The exact role of these enzymes remain to be understood.

D. Hemin Controlled Repressor (HCR) :

This protein kinase, isolated from rabbit reticulocyte lysates or retic lysate, inhibits protein synthesis. Hemin blocks the inhibitory activity of this protein kinase. An inactive form of HCR was activated on warming at 34°C (79). In rabbit retic lysate, it was shown by SDS-PAGE that HCR phosphorylated the small subunit of eIF2 (80). Purified HCR underwent autophosphorylation and at least two molecules of phosphate could be incorporated per HCR subunit. This autophosphorylation which appears to be important for inhibitory activity could be blocked by hemin (81). Both purified and crude HCR has now been shown to exist as a high molecular weight complex of about 15S that undergoes dissociation into the 5.8S HCR via a 8.5S - 9S intermediates. The low molecular weight species was more active than the high molecular weight one. Pretreatment with NEM caused the conversion of all to low molecular weight species. It has been suggested that in lysates the HCR exists in this high molecular weight form. The low molecular weight form has great efficiency of autophosphorylation, which may be the basis for its higher activity (82). However, the activation of inhibitor and the role of hemin is not clear.



E. Casein Kinases :

Two types of casein kinases often coexist in the same

biological material and are distinguishable by physical and chemical properties. The smaller molecular weight (30,000 dalton) species is referred to as CK-S (lately called CK-I) and this class phosphorylates serine residue. The high molecular weight species is called CK-TS (lately called CK-II) and phosphorylates both serine and threonine (83). The two types of casein kinases are separable by ion exchange chromatography. In several instances a quaternary structure has been reported for the enzyme (84, 85, 86) like $\alpha_2 \beta_2$ . Recently, the ATP binding site of CK-II has been identified by affinity labelling and it was shown to reside in the $\alpha$ subunit, showing it to be the catalytic subunit (87). CK-I can use only ATP as its phosphate donor. CK-II is a unique protein kinase which can use both ATP and GTP as phosphate donors (88, 89, 90). It has been shown that the phosphorylation of casein and casein variants by this enzyme is determined by the state of prior phosphorylation of the substrate molecule (91). Both CK-I and II can undergo autophosphorylation (85, 88, 92, 93).

## ROLE OF PROTEIN KINASES

### In Hormone Mediated Response :

A major aspect of protein kinase action in mediating cellular metabolism has been well studied in response to

hormones. Studies with thyroid stimulating hormone indicated that two isozymes, type I and II, were involved in thyroid hormone induced cell growth and differentiation (94). Comparisons between normal and goitrous tissue showed higher activity of cAMP dependent protein kinase in goitrous tissue (95). Insulin action is also mediated through protein kinases. In rat liver plasma membrane, cAMP dependent phosphorylation of three peripheral proteins was triggered by insulin. However, insulin inhibited the phosphorylation of other integral proteins of rat liver plasma membrane (96). It has been suggested that insulin antagonizes the glucagon induced glycogenolysis by decreasing the activation state of cAMP dependent protein kinase (97). It was shown that epinephrine could increase the cAMP content and protein kinase activity (98). There was correlation between the appearance of cAMP dependent protein kinase type II and the activities controlled by gonadotropins (99). Cyclic AMP dependent protein kinases were shown to play an important role in the differentiation and proliferation of mammary glands in response to prolactin (100). A comparative study of the effect of isoproterenol and prostaglandin $E_1$ on perfused rat heart showed that, though both enzymes caused elevation of cAMP and activation of protein kinase, only isoproterenol could cause phosphorylation of protein kinase substrates (101). These evidences indicate that protein kinases are involved in hormone mediated response.

In Neoplastic Transformation :

Protein kinases have been implicated in cancer as well. It was found that in C3H mouse mammary carcinoma cells, there was a marked alteration in cAMP responsiveness for the activation of protein kinase, when compared to normal mammary cells (102). In human retinal cell carcinoma, it was found that the ratio between two kinds of cAMP dependent protein kinases was much higher in the carcinoma cell extracts (103). These enzymes were also present in human gut carcinoma and adenocarcinoma (104, 105). Protein kinases were detected in human colon carcinoma and rectal tumor (106). W265 mammary tumors responded to cAMP treatment by showing tumor regression. In these responsive cells, on treatment with dibutyryl cAMP ($Bt_2$ cAMP) a protein kinase activity translocated from cytoplasm to nuclei, which was not seen in unresponsive cells (107). In Novikoff hepatoma cells, Ehrlich ascites cells and Walter carcinosarcoma tumor specific protein kinases were reported (108). Recent evidences indicate that the viral gene product responsible for the transformation, caused by a number of viruses, is a protein kinase. This protein kinase uniquely phosphorylates tyrosine residues of cellular proteins. Such kinases have been shown in avian sarcoma virus (109), Rous sarcoma virus (110) and feline sarcoma virus (111). Such evidences open up important avenues for understanding the importance of protein phos-

phorylation in neoplastic transformation.

In Transcription :

Transcription and gene activity in nuclei, may be affected by protein kinase through the phosphorylation of chromatin histone, and nonhistone proteins. Most of the nuclear protein kinases are cyclic nucleotide independent. Rat liver protein kinase NII, from liver nuclei, phosphorylating nonhistone proteins, was suggested to cause alteration in their interaction with DNA or other chromatin components and as a result alter chromatin activity (112). A correlation was observed between enhanced RNA synthesis and protein phosphorylation. In resting lymphocytes stimulation by mitogens increased the rate of phosphorylation of nonhistone proteins of chromatin and labelled uridine incorporation into RNA (113). Four different mammalian cell types showed similar protein kinases phosphorylating chromatin proteins (114). In *in vitro* experiments, it has been shown that the regulatory subunit of protein kinase from brain increased the rate of transcription by changing the number of sites for RNA polymerase on chromatin (115). In cultured hepatoma cells, protein kinases were shown to be associated with nucleosomes (116). Chromatin associated protein kinase in soyabean, however, did not show any effect on *in vitro* chromatin transcription by endogenous RNA polymerases (117). From studies in gibberellin treated maize,

a role for protein kinase as a subunit of RNA polymerase II has been postulated (118). Close association with chromatin and phosphorylation of chromatin proteins distinctly indicate that protein kinases may control gene activity.

In Translation :

The role of protein kinases in translation has been most extensively studied in rabbit reticulocyte lysates, *in vitro*. Under conditions of heme deprivation, in this system, a cAMP independent protein kinase is activated which phosphorylates the $\alpha$ subunit of the initiation factor, eIF2 (119). This interferes with the functions of eIF2. The initiation factors eIF3 and eIF4B were also shown to be phosphorylated (120). A ds RNA activated protein kinase was also shown to inhibit ternary complex formation between GTP, eIF2 and met-tRNA and this also operated by phosphorylating the $\alpha$ subunit of eIF2 (121, 122). In this system, however, it was indicated that cAMP dependent protein kinases were not involved in translational control (123). In wheat germ and *E. coli* also cAMP independent protein kinases were shown to be involved in translational control (124). Very recently, a crude fraction in rat liver, which could inactivate amino acyl tRNA synthetase on incubation with ATP, was identified to contain a putative protein kinase(125). Evidence is now available which show that $Ca^{++}$ and phospholipid can also activate the translation inhibitor (HCR) in

retic lysates and cause phosphorylation of the $\alpha$ subunit of eIF2. But the presence of a $Ca^{++}$ and phospholipid dependent protein kinase remains to be shown (126). It is thus established that protein kinases control translation in eukaryotes.

The presence of different classes of protein kinases, their modulation by various substances and the fact that these enzymes are involved in important regulatory mechanisms, makes them an interesting subject for study.