REGIONAL CENTRE FOR BIOTECHNOLOGY

Seminar series

cAMP dependent protein kinase A (PKA): New insights from an old kinase

Malik Keshwani, PhD

HHMI, University of California, San Diego

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Abstract

cAMP-dependent protein kinase A (PKA) is ubiquitously expressed in mammalian cells and regulates various cellular processes including transcription, metabolism, apoptosis and ion channel regulation. PKA exist as an inactive tetrameric holoenzyme constituting regulatory subunit dimer and two catalytic subunits. The Regulatory subunit (PKA-R) binds two molecules of cAMP and unleashes catalytic subunit (PKA-C) which then carries out phosphorylation of various protein targets. The PKA-C is constitutively active and has two phosphorylation sites, a well-studied site in the activation loop (Thr^{197}) and another site in the C-terminal tail (Ser^{338}) for which the role of phosphorylation is unknown. Using *in vitro* and cell culture studies, we show that cis-autophosphorylation of Ser^{338} occurs cotranslationally, when PKA-C is associated with ribosomes and precedes posttranslational phosphorylation of the activation loop Thr^{197}. Ser^{338} phoshorylation is not required for PKA-C activity or formation of the holoenzyme complex; however, it is critical for processing and maturation of PKA-C, and it is a prerequisite for phosphorylation of Thr^{197}. Once Thr^{197} and Ser^{338} are phosphorylated, both sites are remarkably resistant to phosphatases. Using S49 kin minus cell lines that resist cAMP mediated apoptosis and have no solube PKA-C, we show that PKA-C is not phosphorylated at Ser^{338} and as a consequence completely insoluble and inactive. Furthermore, there is significant reduction of PKA-R1α (R1α), which is required for pro-apoptotic Bim-caspase mediated apoptosis on cAMP treatment. We also show that kin minus cells undergo apoptosis on Dexamethasone treatment via caspase independent Apoptosis inducing factor pathway whereas the wild type S49 cells uses Bim-caspase pathway. We also show that cAMP inhibits Ser^{338} phosphorylation and leads to insolubility and improper maturation of PKA-C and provide a mechanism for the apoptosis resistant phenotype of kin minus lymphoma cells.

We revisited role of metal ions in assisting phospho-transfer. Using PKA as a model kinase, with kinetics and structural studies, we found that metal ion serve as electrostatic quenchers of negative charges of ATP so that ATP can bind the negatively charged active site of protein kinase thus facilitating phospho-transfer. We also show that all divalent metal ions assist in phospho-transfer reaction using two different protein kinases. Our data suggests that metal ions do not affect the rate of phospho-transfer but instead just serves as carriers of ATP into and out of the active site of protein kinase.