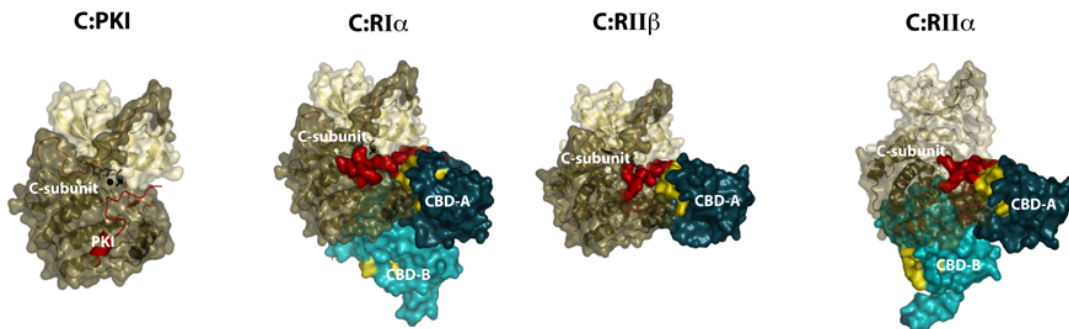


**Dynamics of PKA Regulation . S. S. Taylor, C. Kim, C. Cheng,
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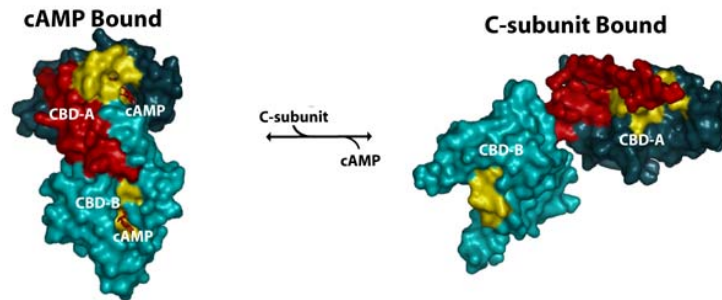
cAMP-dependent protein kinase (PKA), ubiquitous in mammalian cells, regulates processes as diverse as metabolism, development, and memory. The catalytic (C) subunit serves as a structural and biochemical prototype for the protein kinase superfamily and provides a template for understanding the catalytic cycle of phosphoryl transfer. As we move forward in trying to understand this enzyme family which is so fundamental for well-being and is associated with so many diseases, it is essential that we also have a molecular understanding of the mechanisms for activation and for regulation. In the case of PKA regulation is mediated by a family of regulatory (R) subunits designated as RI and RII. These R-subunits are the major receptor for cAMP in mammalian cells and thus serve to link two important signaling events, protein phosphorylation and cellular gradients of cAMP. The dimeric R-subunits bind to two C-subunits in the absence of cAMP to form an inactive holoenzyme complex. Binding of cAMP with high affinity to the R-subunits unleashes the active C-subunits. The R-subunits are comprised of an N-terminal dimerization domain followed by a flexible linker and two tandem cAMP binding domains. The dimerization domains serve as the docking site for A Kinase Interacting Proteins (AKAPs) and colocalize PKA with specific signaling substrates at cellular subsites.

Figure 1. PKA Holoenzyme Complexes



To understand the molecular basis for activation and inhibition of PKA, we have crystallized the C-subunit bound to deletion mutants of three isoforms of the R-subunit, RI α , RII α , and RII β . These structures reveal that all R isoforms dock to a highly conserved surface on the large lobe of the C-subunit although the kinase is in a closed conformation with ATP bound for the RI α holoenzyme and in an open conformation without ATP for the RII α holoenzyme. The RII β subunit bound to C in the presence of AMP-PNP resembles the RI α complex and appears to mimic a transition state intermediate. In contrast to the relatively stable conformation of the C-subunit that serves as a scaffold for protein binding, the R-subunits undergo dramatic conformational changes that include not only a reorganization of the helical subdomains but also a major reorientation of the two cAMP binding domains relative to one another (Figure 2). The linker region which is disordered in the cAMP bound conformation of the R-subunits becomes ordered and docks to the active site cleft of the C-subunit in the holoenzyme complexes.

Figure 2. Conformational Changes Associated With Holoenzyme Formation



The holoenzyme complexes not only redefine the R-subunits as remarkably malleable proteins but also provide novel surfaces that are structurally coupled to the functional state of the PKA. Preliminary surface analysis of holoenzyme complexes found many new sites near the complex interface that can be specifically targeted for drug design. We have developed a high throughput assay for the type I α holoenzyme that can identify both inhibitors and activators that are distinct from ATP competitive inhibitors. We believe that this is a strategy that could have broad applicability to other protein kinases. These holoenzyme complexes, as well as complexes of protein kinases with their protein substrates will be essential for our understanding of this enzyme family.