ABSTRACT

Selective, allosteric regulators alter the conformation and thereby control the activity of cyclin dependent kinase 2 (Cdk2). We used a series of pulsed double electron-electron resonance (DEER) experiments to resolve the degree to which Cdk2 interconverts between the active and inactive states in response to endogenous allosteric modulators (cyclin and phosphorylation) and artificial effectors (ligands and mutations). Our results show that cyclin binding to unphosphorylated Cdk2 only effects a partial shift of the conformational equilibrium to the active state, and that full activation additionally requires phosphorylation of T160 on the activation loop. However, this requirement for phosphorylation of the Cdk2-cyclin complex for a wholesale conformational rearrangement is mitigated by the binding of AMPPNP or dinaciclib in the active site. Furthermore, targeted mutations of the “autoinhibitory hub” of the kinase, either to extend its hydrophobic core or to disrupt it, resulted in a shift of the conformational equilibrium of the Cdk2-cyclin complex to the inactive and active state, respectively. Together, these results suggest that Cdk2, and particularly the unphosphorylated Cdk2-cyclin complex, exists in a delicate balance between conformational states, and that the system is primed for allosteric inhibition that overrides the activating effects of cyclin in the therapy of Cdk2-mediated cancers.