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Evolved to Be Active: Sulfate Ions Define Substrate Recognition Sites of CK2α and Emphasise its Exceptional Role within the CMGC Family of Eukaryotic Protein Kinases

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CK2α is the catalytic subunit of protein kinase CK2 and a member of the CMGC family of eukaryotic protein kinases like the cyclin-dependent kinases, the MAP kinases and glycogen-synthase kinase 3. We present here a 1.6 Å resolution crystal structure of a fully active C-terminal deletion mutant of human CK2α liganded by two sulfate ions, and we compare this structure systematically with representative structures of related CMGC kinases. The two sulfate anions occupy binding pockets at the activation segment and provide the structural basis of the acidic consensus sequence S/T-D/E-X-D/E that governs substrate recognition by CK2. The anion binding sites are conserved among those CMGC kinases. In most cases they are neutralized by phosphorylation of a neighbouring threonine or tyrosine side-chain, which triggers conformational changes for regulatory purposes. CK2α, however, lacks both phosphorylation sites at the activation segment and structural plasticity. Here the anion binding sites are functionally changed from regulation to substrate recognition. These findings underline the exceptional role of CK2α as a constitutively active enzyme within a family of strictly controlled protein kinases.

Keywords: protein kinase CK2; casein kinase 2; CMGC family of eukaryotic protein kinases; constitutive activity; protein crystallography

CK2α is the catalytic subunit of protein kinase CK2 (former name casein kinase 2). Together with CK2β, a non-catalytic anchor protein (also referred to as regulatory subunit) serving as a docking platform for substrates and other binding partners,[1] CK2α associates to a CK2 holoenzyme with $\alpha_2\beta_2$ stoichiometry. CK2 is a pleiotropic and acidophilic serine/threonine kinase with more than 300 substrates described in the literature[2]; its consensus sequence for phosphoacceptor site recognition (S/T-D/E-X-D/E) contains two acidic determinants at the P+1 and the P+3 position, one of which at least must be present in a substrate.[2]

Within the phylogenetic tree of eukaryotic protein kinases (EPK) CK2α is a remote member of the CMGC family branch[3,4] (Supplementary Data Figure 1) and contains an extended insert in the C-terminal domain after helix αG (Figure 1(a)), which is a CMGC-typical landmark. The eponymous members of the CMGC family are the cyclin-dependent kinases (CDK), the mitogen-activated kinases (MAPK), glycogen synthase kinase-3 (GSK3) and the cell division control 2 (CDC2)-like kinases

Abbreviations used: AMPPNP, adenylyl imidodiphosphate; CAPK, cyclo-AMP-dependent protein kinase; CDK2, cyclin-dependent kinase 2; CK2, casein kinase 2; CK2α, catalytic subunit of protein kinase CK2; CK2β, non-catalytic subunit of protein kinase CK2; *hs*CK2α$^{1-335}$, C-terminal deletion mutant of recombinant human CK2α; EPK, eukaryotic protein kinase; GSK3, glycogen-synthase kinase 3; MAPK, mitogen-activated protein kinase; PDB, Protein Data Bank.

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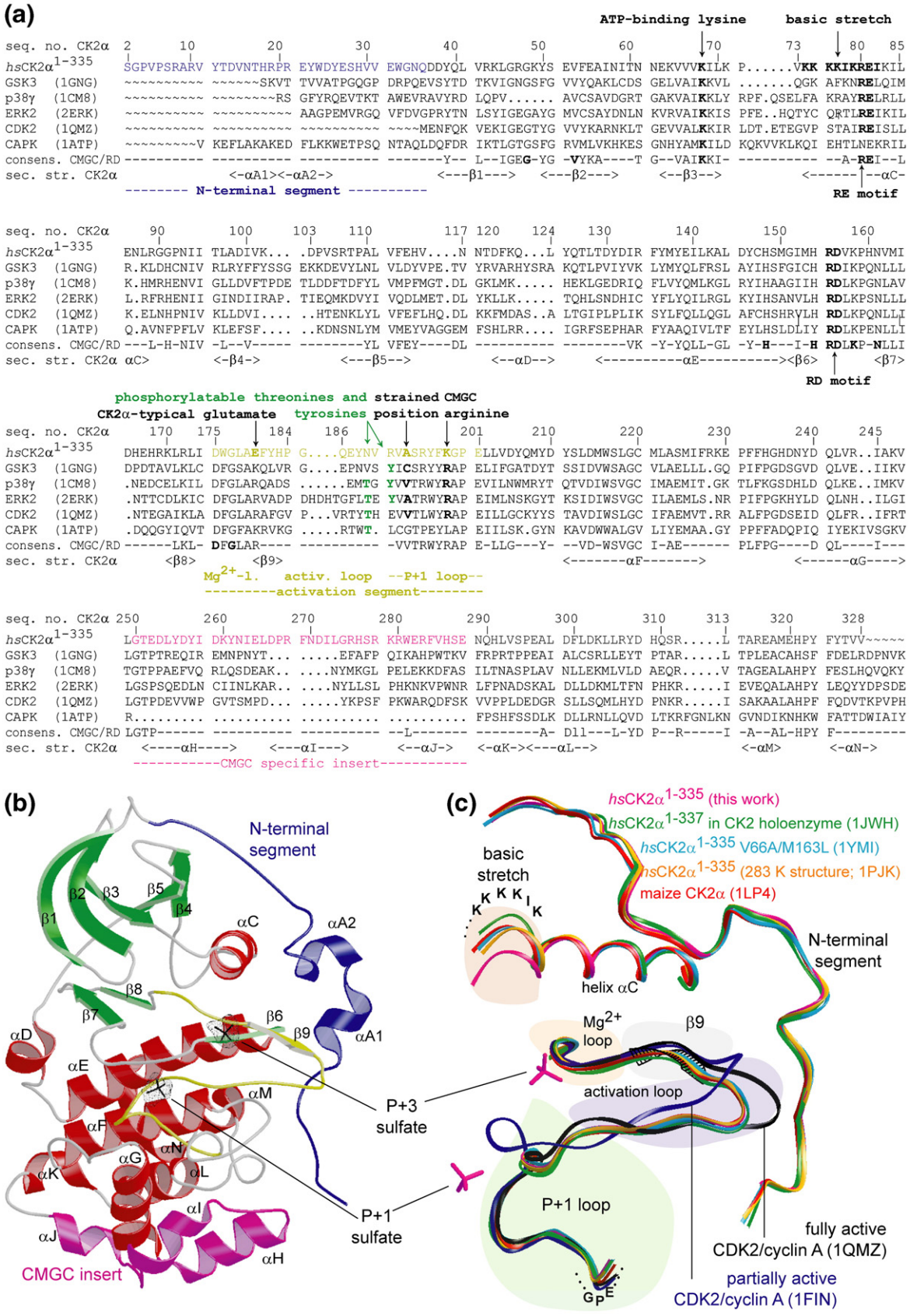

Figure 1 (*legend on next page*)