Protein kinase CK2 – from structures to insights

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Running title

Structural biology of protein kinase CK2

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Abstract

Within the last decade 40 crystal structures corresponding to protein kinase CK2 (former name: "casein kinase 2"), to its catalytic subunit CK2 α and to its regulatory subunit CK2 β were published. Together they provide a valuable, yet by far not complete basis to rationalize the biochemical features of the enzyme such as its constitutive activity, its acidophilic substrate specificity, its dual-cosubstrate specificity or its heterotetrameric quaternary structure. Comprehensive sets of structural superimpositions reveal that both CK2 α and CK2 β are relatively rigid molecules. In CK2 β the critical region of CK2 α recruitment is pre-formed in the unbound state. In CK2 α the activation segment – a key element of protein kinase regulation - adapts invariably the typical conformation of the active enzymes. Recent structures of human CK2 α revealed a surprising plasticity in the ATP-binding region suggesting an alternative mode of activity control.

Introduction

The structural biology of protein kinase CK2 began in 1991 with the crystal structure of cAMP-dependent protein kinase (CAPK) [1], which was the first 3D-structure of a member of the eukaryotic protein kinase (EPK) superfamily [2] to be published. This structure inspired researchers to regard CK2 from a three-dimensional perspective, leading to the construction of homology models [3], the structure-based designing of relevant mutants [4,5] and the formulation of hypotheses with a structural background. This structural approach received a solid basis a decade ago with the crystallographic analyses of the three main CK2 entities - the catalytic subunit CK2 α [6] (Fig. 1A/B), the regulatory subunit CK2 β [7] (Fig. 1C/D) and the heterotetrameric complex of both these components in the CK2 holoenzyme [8] (Fig. 1E).

From a global view, the CK2 α structure contained all the features that one would expect to find based on the already known structures of CAPK [1], cyclin-dependent kinase 2 (CDK2) [9,10,11] and some other EPK enzymes. The CK2 α molecule is made up of a catalytic core composed of two major folding domains (Fig. 1A) harbouring the active site in between. The N-terminal domain is the smaller one and belongs to the (α + β)-type of folding domains. Its constituting element is an antiparallel β -sheet composed of five β -strands. It is accompanied by a single, long α -helix located next to the inter-domain cleft (helix α C, Fig. 1A/B) which is required for function rather than for structural reasons.

Concerning its principle architecture the C-terminal domain of CK2 α is conversely constructed compared to the N-terminal one: it has an α -helical fold, but at the interdomain region it contains two small, but functionally important, two-stranded β -sheets (β 7/ β 8 and β 6/ β 9, Fig. 1A/B). Most helices within the C-terminal domain are conserved among the EPKs with the exception of the small helices α H, α I and α J (Fig. 1A), which constitute an insertion that is absent in CAPK but which is typical for the CMGC-family of EPKs [12]. This “CMGC insert” thus unambiguously identifies CK2 α as a close relative of the eponymous members of the CMGC-family, which include the cyclin-dependent kinases

(CDK), the mitogen-activated protein kinases (MAPK) and glycogen-synthase kinase 3 (GSK3).

Nearly no inferences could be made for the first CK2 β structure [7], due to the lack of proteins with related amino acid sequences not to mention 3D-structures. Hence the central discovery of this structure – a zinc-finger (Fig. 4A) serving as a dimerization motif and resembling a region of the transcription elongation factor TFIIS [13] – came up as a surprise. Apart from this local similarity the global structure of CK2 β revealed a novel fold [7]. Like in CK2 α , the CK2 β monomer (Fig. 1C) consists of a core of two domains (later called the “CK2 β body” in the context of the CK2 holoenzyme [8]); yet, in this case, the N-terminal region constitutes the larger domain and belongs to the α -helical folding type. This domain is followed by a smaller one which contains a β -sheet and harbours the zinc-binding site.

The first CK2 β crystal structure [7] lacked the C-terminal 33 amino acids, which contains the main CK2 α interaction motif. This missing information was provided by the third of the early key structures, the CK2 holoenzyme structure (Fig. 1E) [8]. This structure revealed a tail in the CK2 β monomer (Fig. 1C) that crosses the CK2 β dimer interface to nestle up against both the second CK2 β monomer and the CK2 α subunit (Fig. 1E). The CK2 holoenzyme structure integrated the previous knowledge about the location of the α/β interface on CK2 α [14] and on CK2 β [15]. Moreover, the structure confirmed the already established structural role of the CK2 β dimer as the constituting center of the holoenzyme to which the two CK2 α subunits attach separately and without contacting each other (Fig. 1E).

Quantity and quality of CK2 structures

At the end of 2008 forty crystal structures of CK2 entities were available from the Protein Data Bank [16] (Tab. 1). Nearly half of them were accompanied with the corresponding structure factor amplitudes (experimental X-ray diffraction data). The latter allow the calculation of electron densities

and thus a critical inspection or even a re-refinement of the respective structure. Like all protein crystal structures the CK2 structures contain regions of disorder, anomalies and local errors [17]. Modern crystallography programs like COOT [18] enable also non-expert users to identify and partly even remove such ambiguities provided that the experimental diffraction data are available. In the future the novel quality-focussed PDB deposition policy [19] ensures that any new CK2 structure will comprise both atomic coordinates and diffraction data; the use of both of them is strongly recommended [20].

Particular advances in the growth curve of CK2 structures (Fig. 2) can be identified in the years 2002 and 2007 when several CK2 α structures with ATP-competitive inhibitors were published, reflecting a general tendency in protein kinase structure research. Meanwhile CK2 is regarded as “druggable” kinase [21,22], and companies involved in drug design efforts started to contribute to the structural knowledge about CK2 [23]; thus, it can be assumed that many more CK2 α /inhibitor structures have been solved, albeit not published.

While the structural information about CK2 α bound small molecules is rapidly growing, an inspection of Tab. 1 reveals its limitations concerning the protein matrices. Although CK2 subunits are encoded in all (CK2 α) or almost all (CK2 β) eukaryotic genomes known today – often in several isoforms –, the predominant majority of CK2 structures refers to one particular entity, i.e. CK2 α , to two species, i.e. *Zea mays* and *Homo sapiens*, and to only one paralog within these species. In particular the CK2 holoenzyme is represented only by the above-mentioned low resolution structure [8].

Technical and functional reference structures

A further limitation of the CK2 structures concerns a technical aspect: all but one CK2 structures (Tab. 1) were solved with the molecular replacement method [24] in which an already known similar structure is used as a starting model. This leads to the so-called “model bias” [24], which means that the final structure preserves to a certain degree – depending on the resolution, the amount of local disorder and the care during the structure refinement – features of the initial model. In particular in the

case of low resolution (around 3 Å) molecular replacement structures the expert advice that one “should be very cautious and suspicious of the results” [24] should be taken seriously.

A model bias can be avoided by solving a structure *ab initio* as it was done among the CK2 structures only for the first CK2 β structure [7]. This fact together with the good resolution (1.74 Å) confers the structure of *hsCK2 β ¹⁻¹⁸²* [7] the rank of a technical reference structure for CK2 β . In a functional sense the missing C-terminal segment certainly limits its value; hence, the recently published structure of *hsCK2 β ¹⁻¹⁹³* – a construct that is fully competent of CK2 α -binding – is a useful supplement [25].

Since no *ab initio* structure of CK2 α exists so far, we solved such a structure for this review using an X-ray diffraction data set with optimized anomalous signal (see Raaf et al. [26] for details). We submitted these data to the AutoRigshaw server [27], selected the SAS protocol (“single anomalous scattering on the basis of sulphur”) and determined thus initial phases of the diffracted beams. These phases were then combined with the corresponding high resolution structure factor amplitudes [28] and served as a basis for automated [29] and finally manual [18] model building. The final structure was submitted to the PDB (code 3H30) and represents a technical reference structure for CK2 α . It largely confirms its molecular replacement based pendant (PDB 2RKP) [28] with the exception of some loop regions and side chain conformations.

Like in the case of CK2 β the technical reference structure of CK2 α is not identical with the functional one since the former contains the ATP-binding region in a conformation that is not fully productive. We will show this below, and we will introduce instead the structure of maize CK2 α in complex with an ATP analogue (PDB code 1LP4) [30,31] as the most relevant CK2 α structure in a functional sense. Finally for the CK2 holoenzyme, the predominant carrier of CK2 activity in higher animals, a functionally important structure exists [8] but its technical limitations make a high-resolution update - preferably solved with an *ab initio* technique – highly desirable.

Table 1: CK2 structures available at the Protein Data Bank [16] ranked according to resolution

Protein construct/ designation in the text	Species	Main ligands	Reso- l. [Å]	Exp. data avail- able?	PDB code	Ref.
<i>hsCK2α</i> ¹⁻³³⁵	<i>H.sapiens</i>	3-methyl-1,6,8-trihydroxyanthraquinone (emodin)	1.50	yes	3BQC	[32]
<i>hsCK2α</i> ¹⁻³³⁵	<i>H.sapiens</i>	5,6-dichloro-1-β-D-ribofuranosyl-1H-benzimidazole (DRB)	1.56	yes	3H30	[28]
<i>hsCK2α</i> ¹⁻³³⁵	<i>H.sapiens</i>	adenosine 5'-(β,γ-imido)triphosphate (AMPPNP), sulphate ions	1.61	yes	2PVR	[33]
<i>hsCK2α</i> ¹⁻³³⁵ V66A/M163L	<i>H.sapiens</i>	adenosine 5'-(β,γ-imido)triphosphate (AMPPNP), glycerol	1.66	yes	3BW5	[28,30]
<i>zmCK2α</i>	<i>Zea mays</i>	[5-Oxo-5,6-dihydroindolo-(1,2-a)quinazolin-7-yl]-acetic acid	1.68	no	1OM1	[34]
<i>zmCK2α</i>	<i>Zea mays</i>	5,8-diamino-1,4-dihydroxyanthrachinon	1.70	no	1M2R	[35]
<i>zmCK2α</i>	<i>Zea mays</i>	2-(cyclohexylmethylamino)-4-(phenylamino)pyrazolo[1,5-a][1,3,5]triazine-8-carbonitrile	1.70	no	2PVJ	[23]
<i>hsCK2β</i> ¹⁻¹⁸²	<i>H.sapiens</i>	-	1.74	no	1QF8	[7]
<i>zmCK2α</i>	<i>Zea mays</i>	4,5,6,7-tetrabromo-N,N-dimethyl-1H-benzimidazol-2-amine	1.77	no	1ZOE	[36]
<i>zmCK2α</i>	<i>Zea mays</i>	1,8-dihydroxy-4-nitro-xanthen-9-one	1.79	no	1M2Q	[35]
<i>zmCK2α</i>	<i>Zea mays</i>	4,5,6,7-tetrabromo-benzimidazole	1.81	no	2OXY	[37]
<i>zmCK2α</i>	<i>Zea mays</i>	5,6,7,8-tetrabromo-1-methyl-2,3-dihydro-1H-imidazo[1,2-a]benzimidazole	1.81	no	1ZOH	[36]
<i>zmCK2α</i>	<i>Zea mays</i>	3,8-dibromo-7-hydroxy-4-methyl-2H-chromen-2-one	1.85	yes	2QC6	[38]
<i>zmCK2α</i>	<i>Zea mays</i>	probably benzamidine	1.86	yes	1LPU	[30]
<i>zmCK2α</i>	<i>Zea mays</i>	adenosine 5'-(β,γ-imido)triphosphate (AMPPNP)	1.86	yes	1LP4	[30]
<i>zmCK2α</i>	<i>Zea mays</i>	2-(4-ethylpiperazin-1-yl)-4-(phenylamino)pyrazolo[1,5-a][1,3,5] triazine-8-carbonitrile	1.90	no	2PVL	[23]
<i>zmCK2α</i>	<i>Zea mays</i>	2-(4-chlorobenzylamino)-4-(phenylamino)pyrazolo[1,5-a][1,3,5]triazine-8-carbonitrile	1.90	no	2PVK	[23]
<i>hsCK2α</i> ¹⁻³³⁵	<i>H.sapiens</i>	3-methyl-1,6,8-trihydroxyanthraquinone (emodin)	1.95	yes	3C13	[32]
<i>zmCK2α</i>	<i>Zea mays</i>	probably benzamidine	2.00	yes	1LR4	[32]
<i>zmCK2α</i>	<i>Zea mays</i>	1,8-dihydroxy-4-nitro-anthrachinon	2.00	no	1M2P	[35]
<i>zmCK2α</i>	<i>Zea mays</i>	N-(3-(8-cyano-4-(phenylamino)pyrazolo[1,5-a][1,3,5]triazin-2-ylamino) phenyl)acetamide	2.00	no	2PVN	[23]
<i>zmCK2α</i>	<i>Zea mays</i>	4-(2-(1H-imidazol-4-yl)ethylamino)-2-(phenylamino) pyrazolo[1,5-a][1,3,5]triazine-8-carbonitrile	2.00	no	2PVM	[23]
<i>zmCK2α</i>	<i>Zea mays</i>	-	2.18	no	1JAM	[39]
<i>zmCK2α</i>	<i>Zea mays</i>	4,5,6,7-tetrabromo-2-benzotriazole	2.19	no	1J91	[39]
<i>zmCK2α</i>	<i>Zea mays</i>	adenosine 5'-(β,γ-imido)triphosphate (AMPPNP)	2.20	yes	1DAW	[30]
<i>zmCK2α</i>	<i>Zea mays</i>	guanosine 5'-(β,γ-imido)triphosphate (GMPPNP)	2.20	yes	1DAY	[30]
<i>zmCK2α</i>	<i>Zea mays</i>	N,N'-diphenylpyrazolo[1,5-a][1,3,5]triazine-2,4-diamine	2.20	no	2PVH	[23]
<i>hsCK2α</i> ¹⁻³³⁵	<i>H.sapiens</i>	glycerol	2.30	yes	3FWQ	[40]
<i>zmCK2α</i>	<i>Zea mays</i>	4,5,6,7-tetrabromo-1H,3H-benzimidazole-2-one	2.30	no	1OXD	[37]
<i>zmCK2α</i>	<i>Zea mays</i>	4,5,6,7-tetrabromo-1H,3H-benzimidazole-2-thion	2.30	no	2OXX	[37]
<i>zmCK2α</i>	<i>Zea mays</i>	4,5,6,7-tetrabromo-2-(methylsulfanyl)-1H-benzimidazole	2.30	no	1ZOG	[36]

<i>hsCK2α</i> ^{1- 329E27A/K76N*}	<i>H.sapiens</i>	-	2.40	yes	1NA7	[41]
<i>hsCK2α</i> ¹⁻³³⁵	<i>H.sapiens</i>	adenosine 5'-(β,γ-imido)triphosphate (AMPPNP)	2.50	yes	1PJK	[42]
<i>zmCK2α</i>	<i>Zea mays</i>	3-methyl-1,6,8-trihydroxyanthraquinone	2.63	no	1F0Q	[43]
<i>hsCK2β</i> ¹⁻¹⁹³	<i>H.sapiens</i>	-	2.80	yes	3EED	[25]
<i>xlCK2β</i> ¹⁻¹⁷⁸	<i>X.laavis</i>	p21WAF1 peptide	2.89	yes	1RQF	[44]
<i>rnCK2α</i> ¹⁻³³⁵	<i>R.norvegicus</i>	sulphate ions	3.00	yes	2R7I	---
<i>(hsCK2α</i> ^{1- 337}) ₂ / <i>(hsCK2β</i>) ₂ *	<i>H.sapiens</i>	adenosine 5'-(β,γ-imido)triphosphate (AMPPNP)	3.10	yes	1JWH	[8]
<i>rnCK2β</i> ^{1-193*}	<i>R.norvegicus</i>	-	3.10	yes	2R6M	---
<i>zmCK2α</i>	<i>Zea mays</i>	adenosine monophosphate (AMP)	3.15	no	1DS5	[14]

*In these cases the status of the respective C-terminus is unclear. The indicated C-termini were not introduced genetically but presumably formed by spontaneous degradation.

Global structural comparisons – detection of variable and invariable regions

Enzymes require structurally invariable frameworks for their functions as well as adaptable regions. EPKs in particular are typically regulatable enzymes and exploit a considerable plasticity in some key regions for activity control [45]. Global structural comparisons can reveal such regions and distinguish them from rigid zones. We performed such analyses here in a way that the impact of crystal packing or refinement errors was reduced and the statistical significance of the results improved: first we included only backbone atoms in the calculations, and second we took all available proto-mers rather than selecting single representatives. Such a comprehensive approach is suggested by a recent analysis of the solution dynamics of ubiquitin in which an ensemble of conformers was detected covering all 46 ubiquitin crystal structures available at that time [46]. In other words: the collectivity of all crystal structures of a protein is an approximation of its solution state.

The PDB files listed in Tab. 1 contain 32 subunits of maize CK2 α , 14 chains of mammalian CK2 α and 16 chains of vertebrate CK2 β . For each of these groups we calculated all possible pairwise structural 3D-fits – 91 for mammalian CK2 α , 496 for *zm*CK2 α and 120 for CK2 β - using all main chain atoms for least squares minimization. Afterwards we computed for each sequence position the mean value of the corresponding RMS deviations and plotted them versus the sequence positions (Fig. 3). Average values over the whole sequences – see horizontal lines in Fig. 3 – allow a distinction of conformationally adaptable regions from rigid ones.

We performed a similar analysis for CDK2, a close CK2 α relative from the CMGC family of EPKs (Fig. 3C). In this case nearly 15000 pairwise 3D-fits were included. A comparison of Fig. 3A/B with Fig. 3C illustrates that CK2 α is significantly more rigid than CDK2 (note the different scales on the Y-axes). In particular maize CK2 α (Fig. 3B) obtains an extremely narrow conformational space while mammalian CK2 α (Fig. 3A) shows some interesting local flexibilities to be addressed later.

In general the terminal residues of proteins are regions of enhanced flexibility unless they are particularly fixed like the N-terminal region of CK2 α (especially maize CK2 α) (blue region in Fig. 1B). Therefore, the introduction of artificial termini can cause pseudo peaks. This phenomenon can be seen around positions 180 and 193 of CK2 β (Fig. 3D). The corresponding maxima originate from the fact that many of the CK2 β structures in the PDB were solved with C-terminal truncation constructs ending near position 180 or at His193.

However, the majority of peaks visible in Fig. 3 reveal genuine plasticities of the polypeptide backbones with potential biological significance. At least the highest of them indicate candidate regions that allow the proteins an adaptation to certain environments and functional situations. Moreover there are zones of remarkable conformational invariance (grey shadows in Fig. 3). We will discuss the functional significance of examples from both groups in the following chapters.

Rigid regions

The zinc-binding motif (zinc finger) of CK2 β

The first structure of a CK2 β construct [7] simultaneously provided the first experimental confirmation that CK2 β contains integral zinc ions and a corresponding zinc-binding motif which before had merely been predicted bioinformatically [47]. The central components of this zinc finger are four cysteine residues whose sulphur atoms form a perfect tetrahedral coordination shell for the metal ion (Fig. 4A). These cysteines and their distances in sequence are absolutely conserved and specific for CK2 β proteins throughout the eukaryotic kingdom. The conserved character of this motif in sequence space is the pendant to its conformational invariance (Fig. 3D).

The zinc finger creates a highly efficient dimerization interface for CK2 β [7] (Fig. 1D) and is thus a necessary condition for the formation of the CK2 holoenzyme [8] (Fig. 1E). Disruption of the zinc finger leads to a CK2 β mutant lacking both the ability to dimerize and to coordinate CK2 α

[48]. Due to its mainly hydrophobic character the region around the two-fold rotation axis is a hydrophobic core as normally found within single folding domains. With $540 \text{ \AA}^2$ the interface is relatively small; as a consequence the resulting dimer is an elongated entity (Fig. 1D) with a relatively big surface in relation to its molecular weight. This qualifies CK2 β ideally for its predicted role of as a multi-variant docking partner [49,50,51].

The CK2 α -binding interface of CK2 β

The dimerization of CK2 β sets the stage for the coordination of two CK2 α subunits which is the only of the many protein/protein interactions discussed for the CK2 β dimer [49,50,51] that has been characterized by various *in vivo* and *in vitro* methods including protein crystallography [8], plasmon resonance [52] as well as isothermal titration and differential scanning calorimetry [25]. On the side of CK2 β the necessary interface is created mainly by the helix α F and the C-terminal tail (Fig. 5A) which are rigid elements (Fig. 3D). The CK2 β tail contains a small two-stranded antiparallel β -sheet (strands β 4 and β 5; Fig. 1C); its peptide backbone does not require the contact to CK2 α for folding but is conformationally pre-formed (Fig. 4C) [25]. This is at least true up to His193, while the remaining 22 C-terminal residues of human CK2 β are either only badly defined by electron density or – after Gln205 - completely disordered [8]. Calorimetric [25] and enzymological [15] experiments have shown that the residues after His193 do not contribute significantly to the affinity between CK2 α and CK2 β . The structure of the CK2 β tail was remarkably confirmed by a recent peptide design study that resulted in effective CK2 β antagonists [53].

The spatial neighbourhood of the two CK2 α binding regions does not depend on their proximity in sequence but on the dimer formation since each of them belongs to a different CK2 β subunit. The overall size of the interface established in this way is $832 \text{ \AA}^2$ [8] which is relatively small [54] and consistent with a non-permanent character of the CK2 holoenzyme. This surprising

message of the CK2 holoenzyme structure together with *in vivo* fluorescence imaging results [55] promoted “a new view of an old molecular complex” [56], meaning that the CK2 holoenzyme might be in a dynamic equilibrium with its subunits. However, whether this is really the case under physiological conditions is an open question [57].

The catalytic loop and further key elements of catalysis in CK2 α

Chemically the selective and region-specific transfer of a high energy phospho group from a nucleotide cosubstrate to a protein substrate is a sophisticated process [58]. Recognition, binding and spatial alignment of the two substrates – one of them after all a macromolecule - and stabilization of the transition state require several side chains and backbone regions from both main domains of the catalytic core of an EPK. Consequently a precise spatial allocation of all these elements is a prerequisite of the reaction. It is well-known that these chemical constraints enforce a high conformational similarity of active EPKs [45]. Vice versa if only one of them is not satisfied, an inactive state results enabling very individual ways of EPK inactivation [45].

Unlike CAPK [59], CDK2 [60] and some other EPKs no CK2 α structure of a ternary complex comprising enzyme, nucleotide (plus magnesium or manganese ions) and a peptide substrate was published so far. The best available CK2 α structure to illustrate the arrangement of key residues in the centre of the active site is a binary complex of maize CK2 α and AMPPNP (plus Mg²⁺ ions) [30] (Fig. 4B)^a. In this structure the nucleotide, the Mg²⁺ ions and the surrounding

^a Interestingly, this complex was obtained by soaking of previously grown apo-*zm*CK2 α crystals in a AMPPNP containing solution rather than by co-crystallization. In contrast all co-crystallization experiments with human or maize CK2 α led either to nucleotide ligands with disordered or non-productively orientated triphospho moieties or – in the case of the very first *zm*CK2 α structure [6] – to a complete misinterpretation of a residual electron density. The correction of this error some years later [30] is one of the rare cases that a structure was subsequently revised by the authors.

enzyme matrix are well defined by electron density, and all these components are in equivalent positions as in the ternary complex of CAPK [59].

Several positively charged groups – two Mg^{2+} ions and the side chains of Lys68 and Lys158 – are necessary to balance the negative charge of the triphospho moiety and to align it correctly. To perform this task, Lys68 in turn requires a back stabilization by Glu81 from the helix αC (Fig. 4B). This salt bridge whose two components are highly conserved among EPKs is a necessary condition for and a critical identification of the active state. In inactive CDK2 for instance it is absent because the Glu81-equivalent side chain (Glu51; black side chain in Fig. 4B) is rotated away together with the complete helix αC .

Lys158 is part of the “catalytic loop” that ranges from Arg155 to Asn161 and is conformationally invariable in CK2 α (Fig. 3A/B), in CDK2 (Fig. 3C) and in other EPKs. The central component of this loop is the “catalytic aspartate” which is absolutely conserved among EPKs (Asp156 in CK2 α ; Fig. 4B). The function of this residue’s side chain is the coordination of the nucleophilic hydroxy group of the substrate; possibly it even acts as a catalytic base abstracting the proton from the attacking group and increasing in this way its nucleophilicity [58].

Two further conserved residues (not drawn in Fig. 4B) – Asn161 and Asp175 – are required for magnesium binding and thus for stabilization of the environment of the γ -phospho group. Asp175 belongs to the “magnesium binding loop”. According to a reasonable definition [61] the magnesium binding loop, the strand $\beta 9$, the activation loop and the P+1 loop (Fig. 4B) form “the activation segment” which is crucial for substrate binding and recognition and critically requires the correct conformation for these tasks.

Structural basis of constitutive activity

The term “activation segment” points to a central regulatory role of this region in many EPKs [45].

As key components of cellular regulation and signal transduction EPKs are typically themselves strictly controlled, and this control often depends on chemical modifications (phosphorylations) and conformational changes of the activation segment. The prime example for the chemical and structural basis of a stepwise protein kinase activation was provided by CDK2 that has been structurally characterized in various functional states – among them a monomeric and inactive ground form [9], a partially active form in complex with cyclin A [10] and a fully active form after activation segment phosphorylation [11]. Structural comparisons of these states point to the activation segment and in the helix α C as key elements of structural adaptation. This is illustrated in Figs. 1B and 4B and particularly emphasized by the global conformational analysis comprised by Fig. 3C.

In CK2 α , however, these regions are invariable (Fig. 3A/B), meaning that in all CK2 α structures known to date – irrespective of the species, the crystal packing, the ligands or the quaternary structure – the regulatory key elements adopt the conformations typical for active EPKs. Several structural reasons for this striking rigidity have been presented in the CK2 literature. The most important one is the extensive contact of the N-terminal segment – that is likewise conformationally invariant (Fig. 3A/B) – both to the helix α C and to the activation loop (Fig. 1B) [6]. Thus, the N-terminal segment is functionally equivalent to the cyclin A protomer in CDK2/cyclin A complex and occupies a comparable region of space [6]. The contact region between N-terminal segment and activation segment (strictly speaking a misnomer in the case of CK2 α) is further stabilized by a nearby conserved water cluster with an embedded chloride ion that has been identified recently [26].

Moreover, stabilization of the activation segment in its active conformation is the most plausible explanation for a conspicuous sequence variation in the magnesium binding loop. In most EPKs this loop contains the sequence motif “DFG” compared to “DWG” in CK2 α . The canonical magnesium binding loop with a central phenylalanine is potentially dynamic. In CDK2 for instance the plasticity of the activation segment begins in this region and includes a rotamer change of the central phenylalanine (Fig. 4D). For the inactivation of MAP kinase this phenylalanine residue can

even flip to completely different positions referred to as “DFG-out” conformations [62,63]. In CK2 α , however, the tryptophane substituting the central phenylalanine permits an additional hydrogen bond that disfavours conformational changes (Fig. 4D).

Consistent to these structural features the removal of the N-terminal segment results in a loss of the catalytic activity [64]. In this context it was demonstrated that CK2 β can rescue the activity of these inactive deletion mutants [64]. A possible explanation for this observations is that the activation segment lacking the contact to the N-terminal segment adopts an inactive conformation but refolds to the active form in the presence of CK2 β [64]. Structures of those inactive mutants have been solved neither alone nor in complex with CK2 β so that this hypothesis remains unvalidated.

The strong stabilization of the active conformation of CK2 α by intermolecular constraints provides the rationale of a long-known feature of CK2 [47]: its “constitutive activity”, meaning the independence from external stimuli like (de)phosphorylation, second-messenger binding or coordination of regulatory proteins. What does this mean for the central problem of CK2 research in the last decades – the search for a regulatory mode that works under physiological conditions? The conformational invariance of the activation segment excludes regulatory modes similar to those of CDKs and MAP kinases but it does not necessarily mean that no regulation of CK2 on the level of the protein exists at all. Glycogen synthase kinase 3 (GSK3) for instance – like CDK2 a close CK2 α relative - is rigid in those regions, too, but nevertheless it can be either further stimulated by phosphorylation at the activation segment [65] or downregulated by phosphorylation of its N-terminal region [66]. Thus, also for CK2 it makes sense to look for alternative modes of regulation that do not require alterations in the canonical EPK control elements.

Structural basis of substrate recognition

One of the gaps in our structural knowledge about CK2 is the fact that the enzyme so far could

never be crystallized in complex with a peptide or even a protein substrate. Hence, the structural basis of the well-established fact that CK2 prefers substrates with negative charged residues at positions P+1 and P+3 from the phosphor acceptor site (consensus sequence S/T-D/E-X-D/E) [67] was never unambiguously clarified.

The best structure to fill this gap is based on the C-terminal truncation mutant *hsCK2α*¹⁻³³⁵ to which two sulphate ions were found attached at the activation segment (PDB code 2PVR [33]). Many EPKs – and especially the closest relatives of CK2α within the CMGC family – have anion binding sites in this region; these are often used for regulatory purposes [61], namely for harbouring phospho groups previously attached to a threonine and to a tyrosine phosphorylation site at the activation loop. Frequently significant structural alterations in the activation segment are required to establish these anion binding sites [61]. In other words: phosphorylation, rearrangement of the activation segment and coordination of the inserted phospho groups are concerted events.

A good example for this principle is the MAP kinase p38γ which is phosphorylated twice in its activated form [68] and binds one of the two phospho groups at the P+1 loop and the other in a positively charged pocket at the activation loop (Fig. 4F). In GSK3 both anion binding pockets are preserved but the threonine phosphorylation site was lost during evolution. As a consequence, in the structure of fully active GSK3 only the P+1 pocket is filled by a phospho group bound to the enzyme itself while the second site was found to be occupied by a sulphate ion [69] (Fig. 4G). GSK3 uses this site for binding and recognition of substrates with a primed phosphorylation at the P+4 position [70].

Finally, in CK2α neither of the two phosphorylation sites is present any longer (Fig. 4H). However, as the two bound sulphate ions [33] prove, the anion binding sites are still preserved and no structural rearrangements are required to establish them. Following the line of arguments of the GSK3 case, it is likely that CK2α uses both sites for substrate recognition, a hypothesis fitting nicely to the consensus sequence of CK2 substrates (S/T-D/E-X-D/E) [67]. Accordingly a substrate

peptide with the sequence DSDDD could be plausibly modelled to *hsCK2α*¹⁻³³⁵ in such a way that the P+1 and the P+3 aspartate side chains coincide with the two sulphate ions [33] and that the P+3 aspartate is located near Lys77 which is consistent with previous mutational studies [71]. The model was supported by the detection of chloride ions in the relevant region indicating binding sites for negatively charged groups [26]. For illustration we superimposed this partially modelled enzyme/substrate complex to the enzyme/cosubstrate complex visible in Fig. 4B where the peptide substrate was drawn.

Taken together, while in the MAP kinases the anion binding sites are only formed after full activation and are exclusively used for regulatory purposes, they are pre-formed in CK2α, free from any regulatory function and pure substrate recognition sites. This finding does not only provide a plausible structural rationalization of the enzyme's substrate specificity (at least concerning the micro-environment around the phosphorylation site), moreover it emphasizes its exceptional constitutive activity. Therefore, it was concluded from these results [33] that CK2α probably never adopts an inactive conformation and that any control mechanism of CK2 activity should work on the level of the CK2 holoenzyme. A recently published *hsCK2α*¹⁻³³⁵ structure [40], however, has opened an unexpected further option described below.

The cosubstrate binding site of maize CK2α – structural basis of dual-cosubstrate specificity

The majority of functional key elements - those responsible for catalysis and substrate recognition - are conformationally invariable in CK2α (Fig. 3A/B). Yet in the case of cosubstrate binding and recognition the situation is more complicated: the most important regions for these functionalities – first the ATP-binding loop (glycine rich loop) that connects the strands β1 and β2 (Fig. 1A) and second the interdomain hinge region - are conformationally conserved in maize CK2α but more variable in human CK2α (the modest plasticity indicated in Fig. 3B for the ATP-binding loop of

zmCK2α is largely due to one outlier, the complex structure with ATP-competitive inhibitor emodin [43]). Therefore, we introduce the basic functionality of this region here with maize CK2α and describe its local adaptability in human CK2α and the inferences of this feature later.

Nowadays, the pharmacological relevance of ATP-competitive inhibitors makes the ATP-site to the structurally best explored region of EPKs. This is also true for CK2 so that details of its interactions with inhibitory ligands are described in a separate part of this multi-author review [72]. However, in the case of CK2 there was a special interest in the structural details of the ATP site long before any pharmacological interest: it is known since the 1960ies [73] that CK2 can accept GTP as a phospho donor as efficiently as ATP. The structural basis of this “dual cosubstrate specificity” was elucidated with two comparative complexes of *zmCK2α* with either AMPPNP or GMPPNP [30].

AMPPNP binds to *zmCK2α* similarly as ATP to CAPK (Fig. 4E): the hydrogen bonding potential of the adenine moiety is saturated equivalently, namely by two peptide groups of the inter-domain hinge region (Glu114 and Val116 in the case of *zmCK2α*; Fig. 4E). For GMPPNP, however, this is impossible since the hydrogen bonding potential of guanine differs from that of adenine. It can only be saturated by a shift of the guanine base of GMPPNP along the peptide backbone (Fig. 4E). Interestingly both the triphospho regions and the enzyme matrices are essentially identical in both cases. All adaption work is left to water molecules that are placed as necessary to saturate the hydrogen bonding potentials both of the enzymes and of the respective ligand (not drawn in Fig. 4E). Thus, the rigidity of *zmCK2α* in the ATP-binding cleft which is obvious from the all-against-all comparison (Fig. 3A) emerged already in the early complex structures and was particularly confirmed by an apo-structure [39] that differed only in some chains from the binary complexes.

Fig. 4E suggests that the two nucleo bases of AMPPNP and GMPPNP define a plane.

Obviously, CK2 α allows a certain degree of freedom within this plane for flat (parts of) ligands as long as the hydrogen bonding pattern is preserved. The exact orientation of the plane is defined by its flanking residues Ile66 (Val66 in *hsCK2 α*) and Met163. In a subsequent structure-based protein design work with human CK2 α these two residues were exchanged against their equivalents in CAPK [30]. In the resulting mutant *hsCK2 α* ¹⁻³³⁵V66A/M163L the “adenine base binding plane” was indeed somewhat inclined as it had been predicted before. In this novel orientation the plane apparently lost its 2D-freedom to a certain degree since the original dual-cosubstrate specificity was changed to a clear preference of ATP over GTP [30].

In summary, this mutant of human CK2 α confirmed the concepts about cosubstrate binding obtained on the basis of maize CK2 α . Hence, at that time there was no doubt that the conspicuous rigidity of maize CK2 α around the cosubstrate site was likewise a feature of the human homolog. However, in retrospect it was the structure of the *hsCK2 α* ¹⁻³³⁵V66A/M163L mutant in which for the first time a novel conformation of the interdomain hinge region was observed [30]. At first, this conformation was interpreted as a consequence of the introduced point mutations [30]; only later it was found also without these mutations and thus recognized as an inherent feature of human CK2 α [28] which leads over to the dynamic regions of the enzyme.

Dynamic regions

The β 4/ β 5-loop of CK2 α – structural basis of CK2 holoenzyme formation and non-competitive inhibition

Before we go into the details of the particular flexibility of human CK2 α in the ATP-binding region it is necessary to describe the adaptability in the β 4/ β 5 loop. The two regions are not in direct spatial proximity (Fig. 1A). Yet the gap between them can be filled either by CK2 β [8,14] or by certain small molecules [28] (Fig. 5A) suggesting a structural and functional correlation between the

$\beta 4/\beta 5$ and the ATP-binding loop mediated via the bound ligand.

All loops connecting the β -strands in the N-terminal domain of CK2 α display a somewhat enhanced plasticity as it is typical for loop regions in proteins (Fig. 3A). However, the $\beta 4/\beta 5$ loop is particularly variable. This loop is part of the CK2 α /CK2 β interface; it adopts an extended (open) conformation in the CK2 holoenzyme [8], but a close one, in which it is bent towards the concave surface of the β -sheet populated with hydrophobic side chains (Fig. 5A), in all existing structures of isolated human or rat CK2 α irrespective of the crystallization and crystal packing conditions.

Thus, in mammalian CK2 α an unambiguous coupling of the $\beta 4/\beta 5$ loop conformation and the quaternary structure exists. In contrast in maize CK2 α , both loop conformations occur but without a clear biochemical background, so that in this case the less frequent closed conformation was interpreted as an exceptional artifact of crystal dehydration [36].

Since the closed $\beta 4/\beta 5$ loop conformation is spatially incompatible with the binding of CK2 β (Fig. 5A), an opening of the loop is a necessary condition for the CK2 holoenzyme formation. It was even argued [25] that this conformational change – which is particularly conspicuous in comparison to the aforementioned rigidity of the CK2 β part of the CK2 α /CK2 β interface - might be a driving force for the high affinity between CK2 α and CK2 β (dissociation constant 12.6 nM [25]). This hypothesis is unproven so far, but its background was the observation that the standard free enthalpy of the subunit interaction is mainly contributed by the enthalpy rather than the entropy.

While the $\beta 4/\beta 5$ loop in its open conformation enables CK2 β binding, it is in its closed conformation part of a (partly) hydrophobic cavity. This cavity can act as an alternative binding site for certain ATP-competitive inhibitors like DRB (Fig. 5A), and was therefore termed “remote cavity” [40] in contrast to the canonical ATP site. The binding of DRB to the remote cavity has an inhibitory impact on CK2 α which can be enzymologically distinguished from the ATP-competitive effect of DRB [28] and which is absent if the cavity is impaired and blocked by CK2 β . It is possible

that some recently described non-ATP-competitive inhibitors that were originally discovered as substances to disturb the CK2 α /CK2 β interaction [74] are also bound to the remote cavity because CK2 β removes their inhibitory effect and obviously displaces them from their binding site. Apart from synthetic small molecules natural metabolites can occupy the remote cavity. This was shown for glycerol [28,40] and for acetate ions (K. Niefind, unpublished results) both of which overlap with the flat benzimidazole moiety of DRB.

In summary, the remote cavity is a relatively unspecific binding site at the N-terminal domain of human CK2 α formed in the absence of CK2 β by the closed β 4/ β 5 loop and the hydrophobic surface of the antiparallel β -sheet. It can be occupied by small molecules and this occupation can change the activity of the enzyme or even the oligomerization state. All this suggests a further exploration of the remote cavity and its constituting elements.

Plasticity in the cosubstrate binding region of human CK2 α – a key for CK2 regulation?

In the first structures of human CK2 α in CK2 β -bound [8] and in unbound form [42] the two main regions responsible for ATP-binding – the glycine-rich loop and the interdomain hinge – were essentially similarly folded as in maize CK2 α . In particular the CK2 holoenzyme structure [8], in which two functional states of the ATP-binding site were captured, apparently confirmed the impression of a vastly rigid catalytic subunit: one of the CK2 α subunits contains an AMPPNP ligand while the other one is in the apo-state, but in spite of this difference only small adaptations of the enzyme occur. Moreover the adenine and the ribose part of the AMPPNP ligand are in similar positions as in the functional reference structure for CK2 α (Fig. 5B).

As denoted above the situation changed with the structure of the mutant *hsCK2 α* ¹⁻³³⁵V66A/M163L in complex with AMPPNP [30]. In this and some subsequent structures of human and rat CK2 α an alternative conformation of the interdomain region was detected so that this region is

now among the most adaptable ones (Fig. 3A). In a cluster analysis the set of human CK2 α structures was divided into those with an open (and *zmCK2 α* -similar) hinge region and those with a closed one [32]. Both principle conformations of this region are visible in Fig. 5B with two representatives. Particularly conspicuous is the conformational change of Phe121 which resembles the switch between “DFG-in” and “DFG-out” in the MAP kinase p38 γ [63]: in the closed conformation the Phe121 side chain points to the interior of the CK2 α , but in the open one to the surface.

Which consequences has the closure of the hinge region for ATP binding? On the one hand access to the binding cavity is not prohibited, since both in the complex of *hsCK2 α* ¹⁻³³⁵V66A/M163L with AMPPNP [31] and in the *hsCK2 α* ¹⁻³³⁵/DRB complex [28] the ATP site is occupied (Fig. 5B). However, a comparison with the *zmCK2 α* /AMPPNP complex [30] suggests that the closed hinge region conformation corresponds to a non-productive mode of nucleotide binding since already the position of the nucleotide ribose (and even more that of the triphospho moiety) is far away from the functional state (Fig. 5B).

Hence CK2 α with a closed conformation of the hinge region may represent a partly inactive state of the enzyme. This assumption is supported by a recent structure in which not only the hinge region but also the glycine-rich loop was strongly changed (Fig. 5C/D) and had moved into the ATP site (Fig. 5C) [40]. The crystals for this structure had been grown in the presence of AMPPNP and Mg²⁺ ions but nevertheless no nucleotide was bound. Rather the side chain of Arg47 crosses the ATP-binding cleft, establishes a contact to Asp120 and overlaps with the canonical ribose region of the ATP site (Fig. 5C).

Thus, by a conformational transition the glycine-rich loop changed from an ATP-binding to an ATP-exclusion element. The resulting CK2 α structure is the first one, for which the characterization “inactive” is justified. Significantly the conserved ion pair typical for active EPKs (Lys68 and Glu81 in CK2 α ; Fig. 4B) is no longer present since Lys68 has obtained Asp175 – the

magnesium binding aspartate of the active conformation - as an alternative salt bridge partner.

It should be noted that this is a real “conformation” in a chemical sense, i.e. unlike MAP kinases and other EPKs no covalent modification like (de)phosphorylation is required for the transition between active and inactive forms. Therefore, it is reasonable to assume a conformational equilibrium in solution as indicated in Fig. 6 and to ask which factors may affect this equilibrium.

Possible candidates to support the inactive conformation are small molecules. In the corresponding crystal structure [40] the remote cavity is filled with glycerol, but glycerol itself is no inhibitor of CK2 α so that this occupation is certainly not sufficient to promote the inactive conformation. However, other small molecules - like the non-ATP-competitive inhibitors mentioned above [74] - may be more effective (Fig. 6). Thus, it is possible that CK2 β -free populations of CK2 α whose existence has been reported [75] contain to a significant amount inactive conformations of the enzyme.

In contrast to the favouring effects at least one disfavoured factor of the inactive conformation is less speculative: CK2 β stabilizes the whole β -sheet including the glycine-rich loop from the distal side (Fig. 5A) so that its collapse into the ATP-binding site is prohibited. In the sense of the recently supported [46] conformational selection model of protein/protein interactions [76] this means: while (at least) human CK2 α exists in solution as an ensemble of active and (partially) inactive conformers and is thus not fully competent for catalysis, CK2 β specifically selects and stabilizes the fully active one (Fig. 6). This model may provide for the first time a plausible structural basis of CK2 α stimulation by CK2 β . It will be a proof of this model and an attractive task in the future to identify small molecules or other factors that shift the assumed conformational equilibrium to the inactive side (Fig. 6).

The CMGC-insert of CK2 α – a region relevant for substrate recognition?

The helical insert in the C-terminal domain (Fig. 1A) is a typical but functionally somewhat mysterious feature of the CMGC family of EPKs [12]. For structural stability of the C-terminal domain this region is superfluous, but its existence and conservation indicate a functional relevance. A plausible structure/function correlation for the CMGC insert was shown only for the MAP kinase ERK2 [77] where the CMGC insert can fold in such a way that it stabilizes an inactive conformation of the activation segment.

Moreover it was speculated that the CMGC insert might play a role for substrate docking [12]. In fact in recent years the spatial separation of catalytic function from recognition of substrate proteins has attracted increasing attention in EPK research [78]. It is clear now that the integration of EPKs in complex regulatory networks requires more sophisticated mechanisms of molecular recognition than possible with the close-up range around the active site. While many EPKs have special targetting domains to recognize protein interaction partners, CMGC kinases favour an alternative strategy [78]: they dispose of special docking sites located preferentially on the kinase itself like in the MAP kinase ERK2 [79] or on interaction partners like in the case of CDK/cyclin complexes [80].

The CMGC insert of CK2 α is probably no remote docking site in a strict sense, i.e. there are no indications so far that it recognizes special sequence patterns. Nevertheless it may have subtle positive or negative effects on various CK2 substrates and thus favour or disfavour some of them. The adaptability shown at least by human CK2 α (Fig. 3A; Fig. 5E) is probably helpful in this context. At any rate it helps the enzyme to form different crystal packings which represent a special form of protein/protein interactions.

The puzzling acidic loop of CK2 β – a trigger for aggregation?

Finally the acidic loop of CK2 β with a high negative charge density deserves a note among the

dynamic regions of the CK2 subunits (Fig. 3D). “Flexibility” means disorder in this region; it is so high that - with one exception - each of the available structure is interrupted somewhere within the loop. The only exception is one of the CK2 β chains of the CK2 holoenzyme structure [8], but even there the degree of disorder results in an electron density of such low quality that only a coarse backbone tracing is possible (Fig. 5F).

The very limited order in the region depends on protein/protein interactions imposed by the local environment within the CK2 holoenzyme crystals. Among others the acidic loop touches the P+1 loop of a CK2 α chain belonging to a symmetry related CK2 holoenzyme tetramer (Fig. 5F). This structural detail fits to various data and observations with the CK2 holoenzyme [81], in particular its tendency to reversibly form higher-order aggregates. Since this aggregation is coupled to an inactivation, it was speculated that it might play a significant role for CK2 regulation [81]. It was never definitely proven so far that such CK2 holoenzyme aggregates exist *in vivo*, but at least a strong indication was provided: *in vitro* the aggregation of CK2 holoenzyme tetramers was shown to be correlated to their autophosphorylation, more precisely to *trans*-phosphorylation of CK2 β [82,83] and since CK2 autophosphorylation was indeed detected in cells [84], aggregation should also exist *in vivo*. At any rate, the CK2 holoenzyme crystals show that a suitable molecular environment can impose a partial order in the acidic loop of CK2 β . Vice versa, the acidic loop supports in this way the docking of the CK2 holoenzyme to other molecules or to molecular ensembles.

Conclusions

With the initial key structures of CK2 entities (Fig. 1) the fundamental structure/function correlations about this remarkable enzyme seemed to be comprehensively elucidated. Consequently follow-up work on CK2 structures was primarily application-orientated, i.e. to support the important goal to develop effective and selective inhibitors for pharmacology and chemical biology. Only recently, novel structures have indicated that the conformational space of the enzyme is larger than assumed and in

particular includes inactive conformations (Fig. 6). These findings raise many new questions and may thus open a new round of CK2 structural biology in the future.

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Figures

Fig. 1: Overview of key structures of protein kinase CK2

(A) Catalytic subunit CK2 α from *Zea mays* in complex with AMPPNP and magnesium ions [30,31] drawn in a typical perspective introduced for the first time for CAPK [1]. (B) The same as in (A) but turned by 90° around a vertical axis so that the interaction of the N-terminal segment (blue) to the typical key regulatory elements of EPKs - the activation segment (yellow) and the helix α C - can be perceived. To indicate a possible inactive conformation of these elements they are drawn in black colour as found in inactive CDK2 [9]. (C) Architecture of a CK2 β subunit extracted from the structure of the human CK2 holoenzyme [8]. The two main domains and the extended C-terminal tail that nestles up against the second CK2 β subunit (see part E of this figure) are indicated by different colours. (D) Structure of dimeric *hsCK2 β* ¹⁻¹⁸² [7] - the first structure of a CK2 β construct and the first experimental confirmation of a zinc finger motif. (E) First and so far only structure of a CK2 holoenzyme complex [8]. All pictures were drawn with BOBSCRIPT [85] and Raster3D [86].

Fig. 2: Growth of the knowledge about CK2 structures.

The histogram indicates the cumulative numbers of CK2-related structures deposited in the Protein Data Bank [16].

Fig. 3: Comprehensive conformational analysis of CK2 and CDK2 crystal structures.

All possible pairwise 3D-fits within four clusters of structures - mammalian CK2 α (A), maize CK2 α (B), human CDK2 (C) and CK2 β (D) - were calculated with the program LSQKAB [87] including all main chain atoms. For each residue the least-squares-minimized RMS deviations were averaged over all four non-hydrogen main chain atoms. The resulting mean values were again averaged over all 3D-fits within the respective cluster of structures and plotted against the sequences. A horizontal line in the height of the average over the whole sequence supports the distinction of adaptable from invariable regions. Several rigid zones are indicated by grey patches.

Fig. 4: Rigid regions in CK2 structures

(A) The zinc-finger of CK2 β responsible for dimerization as found in *hs*CK2 β ¹⁻¹⁸² [7]. (B) Catalytic key region in the complex structure of *zm*CK2 α and AMPPNP [30,31]. All important parts are well ordered as indicated by electron density contoured at 1.5 σ . A short substrate peptide – originally modelled to *hs*CK2 α ¹⁻³³⁵ [33] – was drawn with black carbon atoms. Moreover the helix α C of inactive CDK2 [9] with the critical conserved glutamate residue is illustrated after structural superimposition. (C) C α -trace of the CK2 β tail region in the CK2 α bound [8] and unbound [25] case. (D) The exceptional DWG-motif of CK2 α at the N-terminal end of the activation segment. The dotted line indicates the additional hydrogen bond compared to CDK2 whose equivalent DFG-motif was drawn in the active and the inactive conformation. (E) Structural basis of dual-cosubstrate specificity: binding of either AMPPNP or GMPPNP to the hinge region of maize CK2 α . For comparison ATP as bound to CAPK [59] was drawn in black colour. (F-H) The anion binding sites at the activation segment filled by dual phosphorylation (p38 γ ; F), one phosphorylation and an additional sulphate ion (GSK3; G) and two sulphate ions (CK2 α ; H). Parts A to E of this figure were prepared with BOBSCRIPT [85] and Raster3D [86] and parts F to H with BRAGI [88].

Fig. 5: Dynamic regions in CK2 structures

(A) The $\beta 4/\beta 5$ -loop of CK2 α . It contributes in its open conformation (blue) to the binding interface for CK2 β [8] and in its closed conformation (grey) to the remote cavity that can harbour DRB and other small molecules [28]. (B) The interdomain hinge region in two fundamental conformations: the open conformation is found in the functional reference structure for CK2 α (*zm*CK2 α /AMPPNP complex; yellow) [30] and in the CK2 holoenzyme (black) [8], while the closed conformation is represented by the *hs*CK2 α^{1-335} /DRB complex (magenta) [28] and by the complex of *hs*CK2 α^{1-335} V66A/M163L with AMPNP complex (green) [31]. (C) Collapse of the glycine-rich [40] loop changing its function from an ATP-binding element to an ATP-exclusion factor. The black bonds represent the functional reference structure (*zm*CK2 α /AMPPNP) [30]. (D) The glycine-rich loop integrated into the antiparallel β -sheet in a view from the CK2 β dimer. The colour-coding is identical to part B apart from the addition of a blue C α -trace showing the inactive conformation [40]. (E) The major part of the CMGC insert in two typical conformations found in *hs*CK2 α^{1-335} in complex with either DRB (red helices; [28]) or sulfate ions (blue helices; [33]). (F) The acidic loop of CK2 β in its only partially ordered version found in chain D of the CK2 holoenzyme structure [8]. The acidic loop is covered by electron density with a contouring level of 1 σ . Parts A and E of this figure were prepared with BRAGI [88], all other parts with BOBSCRIPT [85] and Raster3D [86].

Fig. 6: A hypothetical working model for CK2 α regulation. According to the conformational selection model of protein interactions [76] CK2 β stabilizes the active conformation but certain small molecules the inactive conformation of CK2 α . The figure was adapted from Raaf et al. [40].

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