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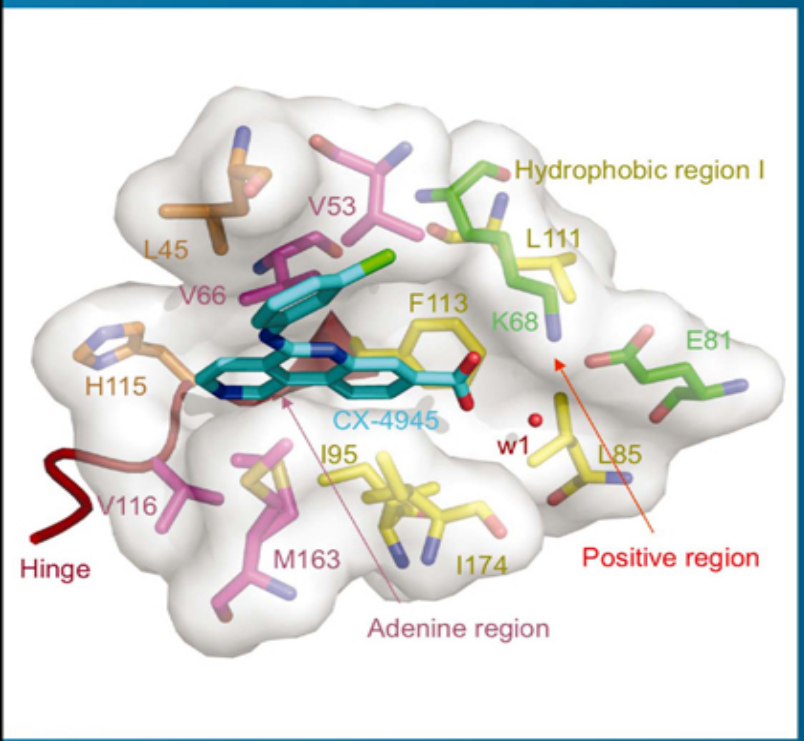
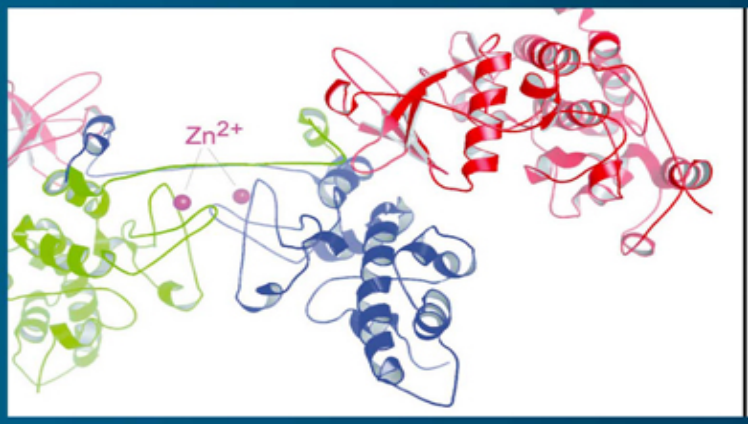
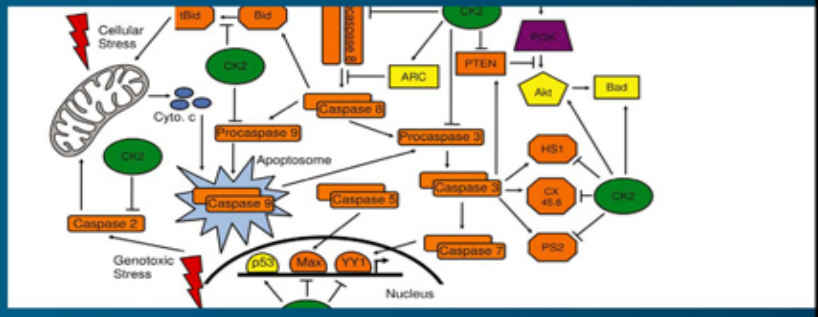
Protein Kinase CK2

Edited by **Lorenzo A. Pinna**







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PROTEIN KINASE CK2

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Cover image: The cover figure provides examples of CK2 peculiar properties by showing, clockwise, starting from left upper corner: the unique butterfly shape of CK2 holoenzyme, composed of two catalytic subunits bound to a dimer of the noncatalytic subunit; the CK2 catalytic subunit pharmacophore occupied by an inhibitor now in clinical trials as an anticancer drug; the convergence of CK2 with caspase pathways; the dorsal axis duplication induced by injecting Xenopus laevis embryos with CK2 mRNAs. Figures are drawn from Chapters 1, 5, and 4, respectively.

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PREFACE

A KINASE FOR ALL SEASONS

In the twilight of his scientific life, the Nobel laureate Edwin G. Krebs became more and more attracted by protein kinase CK2. In a 1999 paper (Mol. Cell. Biochem. 191: 3–12), tellingly entitled “CK2, a protein kinase of the next millennium,” he wrote that such a title was “intended to emphasize the fact that CK2 is such a rich topic for investigation that research involving this enzyme will continue for decades to come.” This statement is one of the justifications for devoting an entire book to an individual member of the human “kinome,” a huge gene family including more than 500 enzymes.

Indeed the long history of CK2, from its early—and in some ways “premature”—discovery in 1954 to the present day, is unique and paradoxical in several respects. CK2 activity was the first example of an enzymatic phosphorylation reaction affecting a protein rather than a small metabolite, leading Eugene Kennedy to coin the term “protein (phospho) kinase” (J. Biol. Chem. 211:: 969–980, 1954). For decades, however, and at variance with other protein kinases discovered between 1955 and 1980, notably phophorylase kinase, PKA, PKG, and PKC, which were immediately recognized to participate in signal transduction pathways, the biological role of CK2 remained obscure. Indeed, its physiological targets remained entirely unknown for many years, its activity being measured *in vitro* with proteins that were not its physiological target, such as casein, leading to it being “misnamed” “casein kinase 2,” a historical name still hinted at by its current acronym of CK2.

The first physiological targets of CK2 were discovered in the late 1970s, causing CK2 to be independently "re-discovered" by a number of researchers working in different areas, for example as a "glycogen synthase kinase 5" (GSK-5) (Cohen P et al. Eur. J. Biochem. 124: 21–35, 1982) and a "Troponin-T kinase" (Villar-Palasi C et al. J. Biol. Chem. 256:7409–7415, 1981), as has been discussed elsewhere (Pinna LA Cell. Mol. Biol. Res. 40:391–399, 1994). Later, by a remarkable "snowballing" effect, the pleiotropy of CK2 eventually came to surpass that of any other individual protein kinase, with more than 300 substrates identified by 2003 (Meggio F and Pinna LA FASEB J. 17:349368, 2003). However, even this number is a huge underestimate of the total number of CK2 substrates that undoubtedly exist, bearing in mind that recent proteomic analyses have revealed that a large proportion of naturally occurring phosphorylation sites in proteins display the unique acidic motif C-terminal to the phosphorylated residue that is recognized specifically by CK2. This suggests that more than 20% of the entire human phosphoproteome may be generated by this individual protein kinase (see Salvi and Cesaro's discussion in Chapter 3 of this book).

The pleiotropy of CK2 is now considered to be just one facet of this remarkable enzyme, its unique feature being its "constitutive" activity, an intriguing property whose structural basis is discussed by Niefind and Battistutta in the first chapter of this book. This is in contrast to many other protein kinases, which are silent under basal conditions and only become active in response to specific stimuli. CK2 seems to always be present in cells in an active conformation, without the need of phosphorylation events to sustain its activity. In this respect, it is therefore quite different from kinases that participate in signaling "cascades." However, to exclude CK2 from participation in signaling pathways would be an incorrect inference,

contradicted by the overwhelming evidence that CK2 impinges on many signaling pathways, but in a unique “lateral” fashion rather than a “vertical” linear manner (see Chapter 5 by Gabriel and Litchfield and Chapter 11 by Ruzzene in this book).

Constitutive activity also underlies another paradox of CK2: many oncogenes encode protein kinases endowed with inappropriate activity or a gain of function mutation. Although this might appear to exclude CK2 from being an oncogene, since no gain of function mutations have ever been reported, nonetheless CK2 is clearly implicated in many cell biology phenomena that are associated with cancer, and the expression and activity of this protein kinase is invariably high in malignant cells compared to untransformed cells. This issue is dealt with in several chapters of this book. An attractive explanation for this apparent contradiction seems to be that diverse neoplastic cells become “addicted” to abnormally high levels of CK2 to such an extent that pharmacological downregulation of CK2 can reverse the tumorigenic phenotype. There are two important consequences of this situation. Firstly, cells where CK2 is abnormally high are “predisposed” to malignant transformation, thus deserving the neologism “oncophilic” cells (Ruzzene et al. Mol. Cell. Biochem. 356: 5–10, 2011). Secondly, CK2 may represent a pharmacological target for the treatment of a wide range of neoplastic diseases. The structures and mode of binding of several inhibitors in complex with CK2 are described in the first chapter of this book, and a potent and selective CK2 inhibitor is now undergoing clinical trials for the treatment of different kinds of tumors as discussed in detail by Drygin in the last chapter of this book.

Another consequence of the constitutive activity of CK2 is that many viruses and other infectious agents have learned how to exploit its presence in the host cell for the

phosphorylation of proteins that are essential to their life cycle. Therefore, CK2 also represents an attractive target for anti-infectious therapies although in contrast to cancer, where a partial downregulation of abnormally high CK2 activity may suffice, the suppression of host cell CK2 activity may have undesired consequences that still have to be evaluated. Other pathologies where an involvement of CK2 is suspected, mostly based on the scrutiny of its protein targets, are neurodegenerative syndromes, cardiovascular diseases, inflammation, and cystic fibrosis as reviewed by Guerra and Issinger (Curr Med Chem. 15:1870–86, 2008). In these cases, however, the roles of CK2 still need to be unravelled, and it is unclear whether any beneficial effects will come from downregulation or upregulation of CK2 activity.

The widespread and continuously increasing interest in CK2 in the scientific community is obvious from even a cursory scrutiny of the literature, the number of paper mentioning “CK2” in their title rising from 94 in 2000, to 159 in 2005, and 329 in 2011. This mainly reflects the increasing numbers of investigators who are inevitably coming across this kinase in the course of their studies. Although the “love affair” of most scientists with CK2 is transient, there remains a hard core group of “CK2 addicted” labs where this topic has been studied for decades, and this community of CK2 investigators meets periodically to discuss their most recent findings and to try to delineate new perspectives in the field. The first conference was held in Heidelberg, Germany, in 1994, followed by other conferences in Villard de Lans, near Grenoble, France (1997), in San Esteban, Chile (2001), in London, Ontario, Canada (2004), in Padua, Italy (2007), and in Cologne, Germany (2010). These international conferences on CK2 have been sponsored and generously supported by IUBMB. It is therefore not

surprising that a book of the Wiley-IUBMB series is now devoted to CK2.

The first part of this book will deal with structural aspects underlying the unique properties of CK2, its specific susceptibility to pharmacological inhibition, and its extraordinary pleiotropy. In the second part, the fundamental role of CK2 in a wide number of biological functions will be illustrated, and the third part will be devoted to the potential roles of CK2 in malignancy, which is providing new strategies and tools to treat neoplasia.

Chapter 1 by Karsten Niefind and Roberto Battistutta provides a thorough and detailed overview of present knowledge about structural features that underlie the enigmatic mode of regulation of CK2 and its susceptibility to a wide spectrum of potent, selective, and cell permeable inhibitors that are invaluable in helping to dissect the cellular functions of this kinase, as well as to counteract its oncogenic role. This theme will be exemplified throughout the book.

Chapters 2 and 3, by Mathias Montenarh and Claudia Götz and by Mauro Salvi and Luca Cesaro, respectively, deal with the pleiotropic nature of CK2 function, by presenting an updated repertoire of its interacting partners and a proteomic analysis that supports the concept that a substantial proportion of the whole human phosphor-proteome is generated by this single kinase.

A global view of the biological role of CK2 from both an embryogenetic and phylogenetic standpoint is provided by Isabel Dominguez and collaborators in Chapter 4, where the phenotypes of CK2 deregulation in model organisms, with special reference to yeast, C. elegans, Drosophila, and mouse are described.

Chapter 5 by Michelle Gabriel and David Litchfield mainly focuses on the unusual mode of operation of CK2 in signaling pathways and on devices by which the apparent

“lack of control” of CK2 can be overcome. In this connection, special reference is made to “substrate level regulation” mediated by hierarchical phosphorylation.

The next three chapters by David Meek, Yoshihiko Miyata, and Olaf-Georg Issinger and Barbara Guerra, respectively, deal with specific and relevant aspects of CK2 functionality, namely its potential role in the regulation of the tumor suppressor protein p53 (Chapter 6), its role in the Hsp90 chaperone machinery, which is essential for the survival of the “onco-kinome” (Chapter 7), and its involvement in cell survival (Chapter 8).

Chapter 9 by Montserrat Pagès and collaborators is entirely devoted to the distinctive properties of CK2 in plants, where unique structural features of the kinase may reflect roles in a variety of specialized functions.

Chapter 10 is an introduction to the implied involvement of CK2 in neoplasia, where David Seldin and Esther Landesman-Bollag summarize studies that have proved that CK2 has the capability to act as an oncogene. They also show that the overexpression of CK2 is associated with reduced survival and with invasiveness of cancer cells.

In a similar vein, albeit from a different angle, Maria Ruzzene provides evidence in Chapter 11 for a “vicious circle” whereby cells sporadically expressing abnormally high levels of CK2 are predisposed to malignancy if an oncogenic mutation occurs, leading to the selective increase of these cells, which in turn are more susceptible than “normal” cells to the cytotoxic efficacy of CK2 inhibitors.

The concept that malignant cells are more susceptible to loss of CK2 activity than normal cells is also dealt with by Khalil Ahmed and collaborators in Chapter 12, whose important message is that CK2 is deregulated in all cancers examined and that its downregulation results in potent induction of apoptosis. The authors also describe recent

progress in targeting CK2 cancer cells in a specific manner, leading to eradication of the cancer.

An overview of the role of CK2 in normal and malignant hematopoiesis is presented by Francesco Piazza in Chapter 13, showing that CK2 is upregulated in a variety of acute and chronic lymphoid and myeloid malignancies and suggesting that this protein kinase could be a suitable therapeutic target in these cases.

The role of CK2 in the progression of breast carcinoma through its control of epithelial cell plasticity is the topic addressed by Claude Cochet, Alexandre Deshiere, and Odile Filhol in Chapter 14, where the authors describe an unbalanced expression of CK2 subunits in a subset of breast tumor samples providing a detailed explanation for the molecular events underlying this process.

In Chapter 15, Denis Drygin provides a thorough and stringent survey of arguments supporting the concept that CK2 is a “logical target” in cancer therapy, especially if its inhibition is combined with chemotherapeutic agents. In that chapter, the efficacy of CK2 inhibitors whose mode of action is detailed at the molecular level in Chapter 1, is highlighted by showing how the “first-in-class” CK2 inhibitors have entered clinical trials. This has demonstrated for the first time that CK2 can be safely and extensively inhibited in humans without unacceptable side effects.

Needless to say, I am enormously grateful to all of the authors for having participated in this editorial enterprise and for having provided such an excellent series of contributions.

I also wish to thank Professor Angelo Azzi, President of the IUBMB Executive Committee, Professor Willy Stalmans, Chairman of the IUBMB Publication Portfolio, and Professor William J. Whelan, Editor-in-Chief, IUBMB Life, for having given me the opportunity to crown my academic career by editing a book devoted entirely to my “favorite” enzyme,

which has monopolized my attention for decades and I hope will continue to keep me busy scientifically in the future.

Special thanks also to Justin Jeffryes, Wiley's Executive Editor, for his encouragement and continuous support, to Anna Ehler for her invaluable help in editorial matters, and to Luca Cesaro for his help in collecting and assembling the authors' contributions and for preparing the cover figure of the book.

Lorenzo A. Pinna

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Part I

Molecular and Structural Aspects