Multi-author Review
Protein Kinase CK2 in Health and Disease

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Protein kinase CK2: An ugly duckling in the kinome pond

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Introduction (Part of a Multi-author Review)

Nowadays, protein kinases are universally considered signaling molecules *par excellence,* and the 'kinome' has become the Eldorado of pharmaceutical companies in search of druggable targets. However, the discovery of the first protein kinases, more than 40 years ago, was the serendipitous outcome of an ingenious biochemical oddity. In fact, the authors of the first report describing an enzyme able to catalyze the transfer of phosphate from ATP to a protein – Eugene P. Kennedy and his student George Burnett – coined the neologism (at that time!) 'protein (phospho) kinase' and reasoned that casein, one of the very few phosphoproteins known at that time, might have a special propensity to become phosphorylated. Accordingly, they successfully used casein as an artificial substrate to detect protein phosphorylating activity in tissues, notably liver, where casein is not present at all [1].

Later on it was shown by others that such a protein (casein) kinase activity was due to two distinct enzymes, presently termed CK1 (an independent branch of the 'kinome', composed in humans of six isoforms) and CK2, the subject of this multi-author review. This latter enzyme exists with two catalytic subunit isoforms, not belonging to any of the major branches of the kinome, yet phylogenetically close to the CMGC group, composed of the CDK, MAPK, GSK3 and CLK families [2].

Two years after the accidental discovery of CK1/CK2, the first physiologically meaningful protein kinase was identified: this was phosphorylase kinase, responsible for the phosphorylation and activation of phosphorylase b [3]. This finding paved the road toward the discovery of PKA (the main 'activator' of phosphorylase kinase) and several other second-messenger-dependent protein kinases – PKG, PKCs, CaCaM-PKs etc. – an unfortunate circumstance for CK1 and CK2 , which, in contrast, were devoid of any apparent regulatory role and whose biological functions remained a matter of conjecture for decades.

The first physiological substrates of CK2 started being identified only in the late seventies of the past century, more than 20 years after the discovery of CK2 itself. Incidentally, for a while early identification of CK2 protein targets made the issue even more confusing than it was before, since CK2 was independently 're-discovered', e. g. as one of the enzymes responsible for the phosphorylation of glycogen synthase, namely 'glycogen synthase 5' (GSK5) [4] and as 'Troponin-T kinase' [5] (for a detailed historical outline, see [6]). Later, as in a snowball effect, an increasing number of proteins phosphorylated by CK2 underwent detection: these were already almost 160 in 1997 and >300 in 2003 [7]. This is still a ridiculous underestimate if we consider that a recent WebLogo analysis performed on a database of 10 899 naturally occurring phosphorylated sites reveals that 2275 of these (>20 %) display the unique acidic pattern that unambiguously identifies CK2 sites [8].

Detection of more and more numerous CK2 targets, in conjunction with the availability of specific antibodies

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and of very specific peptide substrates suitable for reliable CK2 quantification in crude extract and, more recently, the development of fairly selective cell-permeable inhibitors, have made it possible to at least partially overcome the main 'handicap' of CK2, i. e. its lack of known physiological regulators. They also lead to the unanticipated conclusion that CK2 indeed is a 'master kinase' controlling the activity and/or the lifespan of many other kinases and exerting decision power over cell fate, as will be illustrated in this multi-author review. Thus, the most neglected and disclaimed kinase of the past has evolved into an appealing subject of investigation: the biochemical version of the ugly duckling tale!

Looking at the extreme variety of its targets and partners one should conclude that all or nearly all cellular functions are more or less directly subjected to modulation by CK2. In particular, CK2 appears to play a central role in the regulation of gene expression and protein synthesis/degradation as a mediator of stress stimuli and as a powerful survival agent whose general strategy is to counteract programmed cell death by impinging at different levels on the complex apoptotic machinery.

There are several other cellular processes in which CK2 function appears to play an important role. CK2, for instance, is implicated in various phases of viral infection and replication with a large number of different viruses that impact human and animal health. CK2 is also a participant, together with CK1, in the control of biological clocks. CK2 has been found to act as an ectokinase, being responsible for the phosphorylation of several extracellular proteins.

How can this multi-faceted role as a global regulator of cellular function be reconciled with the apparent lack of control of CK2 itself, whose catalytic subunits (α and/or α') are constantly in the active conformation either alone or combined with the regulatory β subunits, is a puzzling question still awaiting a definite answer. It is possible that conventional modes of regulation, as they are known to operate in the majority of protein kinases, are entirely replaced in the case of CK2 by supra-molecular dynamic association and microcompartmentation devices (see the report by Filhol and Cochet in this multi-author review) or even that physiological ligands are still escaping detection which have the potential to affect CK2 activity by interacting with a binding pocket recently identified on the CK2α surface, close to the ATP binding site [9]. A surprising plasticity found in this region is discussed in the report by Niefind et al. (this issue).

There has been ample discussion regarding the role of CK2β in the regulation of CK2 activity. There are some rather important facts about the effect of this subunit. CK2β has the capacity to modify the activity of the catalytic subunit depending on the nature of the substrate. With most known substrates, CK2β is stimulatory, but with a growing number of other substrates CK2β can drastically inhibit CK2 phosphorylation. This capacity to regulate the activity of CK2α is also applicable to other protein kinases that are able to bind CK2β, such as A-Raf, c-mos and Chk1. Recently, it has been shown that a chimera which combines a large part of the CK1α kinase with the amino-terminal region of CK2α that is responsible for binding CK2β can be stimulated by this subunit [10]. CK2β also causes a significant increase in the heat and proteolytic stability of CK2α and provides 'docking sites' that bind substrates or scaffold proteins. CK2β also appears to be exported to the external side of the cell membrane [11].

Regardless of the possibility that CK2 is endowed with peculiar, subtle modes of regulation, little doubt is left that its high 'constitutive' activity and lack of any clear-cut mechanism of downregulation provide the main argument to explain its implication in a variety of diseases with special reference to neoplasia [12]. Although in fact oncogenic mutations of CK2 have never been reported, CK2 is abnormally high, both in terms of protein and activity, in a wide variety of tumors. This, together with several other observations related to the potential of elevated CK2 to impose unscheduled cell survival, to nullify the efficacy of chemotherapies and to enhance the multi-drug resistance (MDR) phenotype underlies the emerging concept that high CK2 levels create a cellular environment favorable to the establishment and to the enhancement of the tumor phenotype, rather than being itself a cancer promoter [13].

An appealing possibility in this respect would be a global implication of CK2 in 'cancer addiction' whereby cancer cells are often crucially dependent on ('addicted to') the continued activity of overexpressed (onco)genes for the maintenance of their malignant phenotype [14]. A plausible hypothesis would be that addiction to high CK2 levels represents a common denominator of several different kinds of tumors whose malignancy could be effectively counteracted by lowering abnormally high CK2 levels below a critical threshold. This, by the way, would be quite compatible with the caveat that in principle CK2 cannot be fully and stably knocked down, being essential to survival (see Dominguez et al. in this issue).

Not surprisingly, therefore, considerable effort has been expended to develop potent and selective cell-permeable CK2 inhibitors (reviewed by R. Battistutta in this issue). For the time being, the most remarkable breakthrough in this field has come from the recent

announcement (5 January 2009) that a Cylene Pharmaceutical CK2 inhibitor, CX4945, has initiated phase I clinical trials in patients with advanced solid tumors, Castelman's disease and multiple myeloma. The happy end of the ugly duckling tale?

The present multi authors review comprises seven contributions that cover all-important structural and functional aspects of CK2 in normal and in tumor cells. The first paper, by K. Niefind, J. Raaf and O.-G. Issinger, provides a thorough structural insight into CK2, highlighting unique features of this kinase and suggesting new clues for its enigmatic mode of regulation.

The following contribution by N. A. St-Denis and D. W. Litchfield is an overview of the role of CK2 in the regulation of cellular processes, with special emphasis on its implication in the cell cycle, transcription, survival and virus infection. Based on the idea that CK2 may display a 'lateral' rather than 'vertical' means of pathway intervention [7], these authors suggest that CK2 is a key regulatory linker between cellular processes.

The role of CK2 in the transduction of stress signals and a dynamic view of how signaling-dependent subcellular targeting may surrogate the lack of stringent regulation forms the matter of the third contribution by O. Filhol and C. Cochet.

Y. Miyata shows in the fourth review why CK2 deserves being considered a master kinase, controlling through the chaperone system a complicated network of other signaling molecules with special reference to oncogenic protein kinases, the so-called oncokinome. In the following review by D. C. Seldin and co-workers, the tumorigenic potential of CK2 is further delineated as intimately connected with its fundamental role in development. Special attention is devoted to the implication of CK2 in the Wnt and NF-kB pathways, the resulting message being that 'inhibiting CK2 could be useful in treating cancer, but dangerous to developing organisms'. This issue of 'CK2 and cancer' is thoroughly dealt with by K. Ahmed and his co-workers in the sixth review, highlighting the potential of chemical inhibitors of CK2 as anticancer drugs and proposing an ingenious device for their specific delivery to just malignant cells, thus sparing normal cells.

An exhaustive overview of CK2 inhibitors and a structural insight into their mode of action is presented by R. Battistutta in the final review, providing a feedback link to the first contribution (Niefind et al.) by showing how detailed structural information can be exploited for both gaining additional information about CK2 function and developing new tools to counteract its pathogenic effects.

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