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Evolution, dynamics and dysregulation of kinase signalling

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Protein kinases are central regulators of most eukaryotic cellular processes. While kinase signalling has been studied for decades, only through recent advances in mass spectrometry have we been able to identify phophosites in large scale and quantify their regulation across conditions. These advances are challenging our understanding of kinase signalling and shedding light into how these systems have evolved. Kinase substrate specificity appears to be strongly conserved but their target phosphosites diverge at a very fast rate. However, less is known about the functional consequences of such changes and the fraction of phosphosites that are crucial for organismal fitness. A better understanding of these evolutionary processes will facilitate the study of disease related genomic alterations that target these signalling circuits.

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Introduction

Cells constantly have to sense and adapt to changes in the environment and over decades of research we have gained an understanding of what are the molecular mechanisms that work together in what can be defined as cell decision circuits or systems. In a stereotypical and simplified view of such systems from a human cell, a change in the environment is first sensed by membrane receptors such as G-protein coupled receptors [1] or receptor tyrosine kinases [2]. Binding of a trigger molecule results in the activation of the receptors that will lead to the regulation of 'downstream' protein kinases that can modify other proteins by the post-translational addition of phosphate groups to serine, threonine and tyrosine residues. These phosphorylation sites (or phosphosites) can alter the function of proteins by modulating their interactions, localizations and activity. Ultimately, the sum of all of the triggered changes will adapt the cell to the new conditions. The detailed characterization of different molecular aspects of this process has been on-going for decades and has been reviewed extensively [3,4]. More recently with the advances in mass spectrometry based proteomics our knowledge of kinase signalling has been expanding at a high rate. Over the past 5 years the number of identified phosphosites has grown at a tremendous pace. For human alone over 90 000 phosphosites have been identified in single studies [5] and these technological developments have allowed for the unbiased analysis of condition dependent phospho-regulation as well as cross-species comparisons. Such advances are shedding new light on our understanding of how kinase regulatory networks work and how the cell achieves specificity of responses. We review here our current understanding and open challenges in these areas with a specific focus on how kinase signalling changes during evolution or in disease.

Evolution of kinase target specificity at the active site

The fidelity of cell systems control depends on the regulated phosphorylation of a restricted set of substrates for each protein kinase [6,7]. This specificity is defined by the biophysical interactions between the kinase and the substrate at the active site [8–10], and for some kinases also by allosteric 'docking' interactions with the substrate [11,12]. Contextual factors (kinase–substrate co-expression, co-localisation, and adaptor/scaffold binding) are also important in determining whether the kinase and substrate are likely to bind *in vivo* [13,14]. Of these, it is the active site specificity that is the most amenable to kinome-wide evolutionary analysis given the conserved structural fold of the eukaryotic protein kinase (ePK) domain [15].

The most striking difference between kinases in respect to their specificity is the identity of the phosphoacceptor — serine, threonine or tyrosine. Kinases present in the ancestor of all eukaryotic species most likely phosphorylated primarily Ser/Thr given that dedicated tyrosine kinases have been found only in animals, choanoflagellates, and the filasterea. Therefore these kinases must have arisen more recently than Ser/Thr kinases [16]. Recent phosphoproteomic studies in a close unicellular relative of animals — *Capsaspora owczarzaki* — have suggested a possible ancestral role of tyrosine phosphorylation in the temporal control of cell type transitions that

may have been later co-opted for the spatial control of multicellularity [17•]. Kinase specificity can also be determined by the residues directly flanking the phosphoacceptor (proline+1, arginine−3, arginine−2, among others). However, little research has been conducted on the evolution of the specificity determining residues (SDRs) in the kinase active site that shape these preferences. More emphasis has been directed so far towards identifying SDRs from kinase–substrate co-crystal structures [18,19] and from sequence alignments of kinases with known specificity [20–22]. Still, these studies have yielded some evolutionary insight such as the subtle differences in substrate binding between CMGC kinases and other Ser/Thr groups (such as AGC and CAMK) [18] or the common mode of determination for the arginine−2 preference by the distantly related AGC and STE groups [23]. A more comprehensive mechanistic understanding of kinase specificity is currently hampered by the limited diversity of kinase–substrate co-crystal structures present in the PDB.

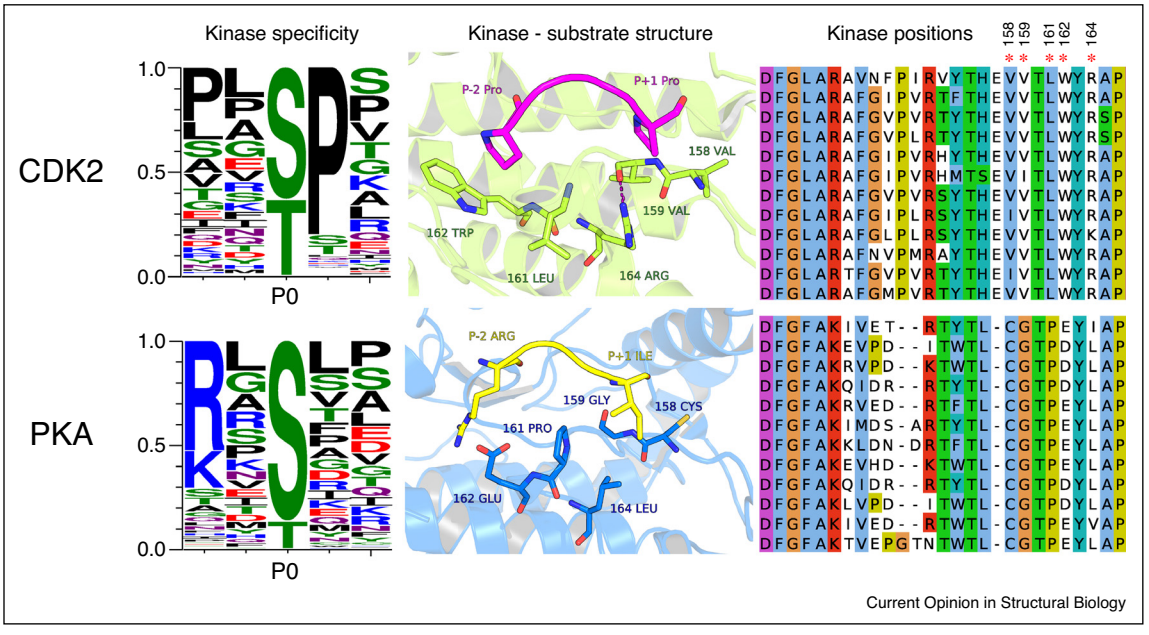
Although the evolution of kinase specificity has not been extensively studied we can hypothesize that orthologous kinases should tend to have conserved specificities. Given that each kinase will often target tens to hundreds of target sites, changes in target recognition would have a detrimental impact. In support of this hypothesis human kinases have been shown to partially or fully complement their yeast orthologs in many cases. This has been shown for example for the yeast kinases CDC28, HOG1, CDC15, MPS1, CAK1, and HRR25 [24,25•]. Additional support can be seen through the analysis of SDR residues in orthologous kinases. For example, we aligned orthologous kinase sequences for the human CDK2 and PKA kinases for which we can model the target site preferences (Figure 1, left and centre). As seen in the sequence alignments (Figure 1, right), important SDR residues in these kinases tend to be highly conserved within orthologous sequences but show striking differences across kinases with different preferences. It is then likely that different kinase target preferences have been created by mutations after kinase gene duplication events.

The extent to which homologous kinases differ between species remains an open question. A detailed study by Howard and colleagues [26] demonstrated experimentally that yeast Ime2 kinase differs in specificity at the +1 position from its mammalian counterparts, and that the likely ancestor was intermediate in preference from the two extant kinases. Sequence-based predictions and experimental methods for kinase specificity determination in additional species could be leveraged in the future to address this issue more systematically.

Very fast divergence of protein phosphorylation may result in non-functional phosphosites

In the past 10 years the comprehensive identification of phosphosites has allowed for studies of their conservation.

Figure 1



Conservation of binding site residues across protein sequences of orthologs of CDK2 and PKA kinase. The kinase specificity preferences for positions −2 to +2 relative to the phospho-acceptor residue were built based on known substrate phosphosites of these kinases in human. Some of the residues important for recognition of these positions are highlighted in the structural model and the conservation at the sequence level shown on the right. Marked with a red asterisk in the alignment are residues that are conserved within orthologs of each kinase but different across the two kinases. These are more likely to be relevant for determining specificity differences across the two kinases.