The extracellular kinome

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The evidence for an extracellular kinome

The first protein kinase was described in the 1950s [1,2]. It was called phosphorylase kinase (PhK), and in the coming years it was found to act as an intracellular relay that could connect extracellular stimuli to the intracellular breakdown of glycogen through phosphorylation and activation of glycogen phosphorylase. Since the discovery of PhK, more than 500 human kinases have been identified [3], and their diverse roles in intracellular signaling are well known. While our understanding of intracellular phosphorylation is growing at a seemingly limitless rate, our understanding of extracellular phosphorylation and its role in signaling is virtually nil by contrast. This is somewhat surprising, since as early as the late 1970s, and continuing into the early 1990s, there were numerous reports of extracellular protein kinase activity detected in a variety of different cell culture systems [4]. In myoblasts, for instance, extracellular protein kinase activity and surface bound phosphoproteins were not only discovered, but evidence suggested that these properties might be required for myotube formation [5–9]. The term ecto-protein kinase was given to these enzymes, although in some cases a finer distinction was drawn between ecto-protein kinases that were associated with the exterior surface of the cell and exo-protein kinases that were present in conditioned media. In myoblasts and the other cell types studied, the identity of these extracellular kinases was never clearly defined, but it may have seemed like only a matter of time before a large number of ecto- and exo-protein kinases were unambiguously described and their role in extracellular phosphorylation delineated in much better detail. Progress was not rapid, however. As of 2005, it had been definitively shown that protein kinase A (PKA) and casein-kinase II (CKII) could be secreted from cells [10,11], but their functions outside of the cell remained a mystery. In 2012, the atypical kinase Fam20C was shown to localize to the Golgi lumen prior to secretion into the media of cultured cells [12]. Fam20C was also shown to phosphorylate several extracellular proteins, including notably casein, as well as proteins involved in biomineralization. Along with some additional data, a role for Fam20C in proper bone development was suggested, although what phosphorylation of its extracellular substrates does is still to be determined. Although this may not seem like much detail, Fam20C represents the best-characterized extracellular kinase. It will be fascinating to see how these discoveries on Fam20C and the other known extracellular kinases are further fleshed out in the coming years, and what we know is surely just the small tip of a very large iceberg as hundreds of extracellular phosphoproteins have already been detected from limited proteomic screens [13,14].

How a kinase becomes extracellular

There are several ways that a kinase may find its way outside of a cell. As with Fam20C, it may be a Golgi-resident kinase secreted from the cell, with the ability to phosphorylate proteins within the Golgi that are also destined for secretion, or it may be able to phosphorylate extracellular proteins following exocytosis. There are two dozen human kinases with predicted secretion signals [15], suggesting many kinases may find their way into the extracellular space through this route. Membrane damage is a second way that cytoplasmic kinases may end up outside the cell. This could be much more than just a circumstantial consequence of membrane injury as the release of a kinase in such a scenario and its phosphorylation of membrane and extracellular proteins could be a critical part of the repair process. A permanent compromise of membrane integrity, as occurs during necrosis, is a third way that kinases, both cytoplasmic and nuclear, could end up outside of the cell – technically outside of neighbouring and still living cells. Such “lysed” kinases could phosphorylate...
proteins on adjacent cells, promoting their own cell death or provoking a protective reaction. Lysed kinases could also potentially phosphorylate proteins on macrophages, initiating a polarized response towards the ruptured cell. A fourth way for a kinase to end up outside of a cell is as part of a multi-domain membrane protein. In this regard, the extracellular domain of the CD4 receptor has been reported to have in vitro kinase activity [16]. This region of CD4 would be classified as an atypical kinase domain, and although there are no known membrane proteins with typical kinase domains in their extracellular regions, there may be more membrane proteins with atypical kinase domains that have yet to be discovered.

The functions of an extracellular kinase

Intracellular protein phosphorylation is known to regulate protein conformation and protein-protein interactions, and it is natural to expect extracellular phosphorylation will regulate similar properties on target proteins. For example, phosphorylation of the extracellular ligand-binding domain (LBD) of a receptor could regulate ligand binding. Several phosphorylation sites have been detected in the LBD of the epidermal growth factor (EGF) receptor [17], and one of these target residues, tyrosine 113, is located at the binding interface with EGF [18]. It is also possible that phosphorylation of an extracellular residue could lead to a conformational change within the intracellular portion of a transmembrane protein and/or dimerization, much in the same way as ligand binding to a receptor can. Phosphorylation of membrane proteins could also affect protein-protein interactions between cells, and, in fact, this has already been shown. Cadherins are cell adhesion proteins and numerous phosphorylation sites have been detected in their extracellular domains [17]. In drosophila, Fat and Dachsous are cadherins that interact between cells to regulate the Hippo signaling pathway [19], and their interaction is regulated by at least one phosphorylation site present in the third cadherin domain of Fat [20]. This site is targeted by the kinase Four-jointed, which localizes to the lumen of the Golgi and can be secreted as well [21,22]. Further characterization is needed, but the data is consistent with Four-jointed phosphorylating Fat in the Golgi and/or extracellular space, thereby regulating Fat’s interaction with Dachsous on adjacent cells, and ultimately influencing Hippo signaling.

Although very little is known about phosphorylation, kinases and signaling outside of the cell, the pieces of data we have on kinases such as Fam20C and Four-jointed, combined with ever-growing phosphoproteomic data, are consistent with an extensive, dynamic and largely unknown world of extracellular signaling. Most of the extracellular signaling scenarios we have proposed are hypothetical with only tantalizing evidence to support them, but in the coming years it seems likely that these and similar scenarios will become bolstered by more concrete data. Of course, phosphorylation is only one type of post-translational modification (PTM) and the potential for other extracellular PTMs means that kinases and phosphorylation may only be a small part of a much bigger and more complex picture. It is exciting to think of the discoveries that are to come.

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References

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