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Review

# Tyrosine kinase signaling and the emergence of multicellularity

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ABSTRACT

Tyrosine phosphorylation is an essential element of signal transduction in multicellular animals. Although tyrosine kinases were originally regarded as specific to the metazoan lineage, it is now clear that they evolved prior to the split between unicellular and multicellular eukaryotes (≈600 million years ago). Genome analyses of choanoflagellates and other protists show an abundance of tyrosine kinases that rivals the most complex animals. Some of these kinases are orthologs of metazoan enzymes (e.g., Src), but others display unique domain compositions not seen in any metazoan. Biochemical experiments have highlighted similarities and differences between the unicellular and multicellular tyrosine kinases. In particular, it appears that the complex systems of kinase autoregulation may have evolved later in the metazoan lineage.



Protein phosphorylation is a sine qua non of signal transduction in both prokaryotes and eukaryotes. While prokaryotic phosphorylation is dominated by phospho-histidine signaling, phosphorylation of serine, threonine, and tyrosine predominates in eukaryotes [1,2]. Eukaryotic tyrosine phosphorylation is catalyzed by a specific group of protein kinases (designated TK-group kinases in this review). The emergence of these enzymes appears to be a relatively recent innovation in the history of life. TK-group kinases are absent in prokaryotes, although some prokaryotes have unique tyrosine kinases (designated BY kinases) that are related to the nucleotide triphosphatase superfamily [3]. Eukaryotic-like tyrosine kinases are also absent in plants, in yeast and other fungi, and in the slime mold *Dictyostelium discoideum* (Fig. 1) [1,4]. Thus, the view emerged that TK-group kinases were metazoan-specific signaling enzymes. Tyrosine kinases play essential roles in the regulation of growth and differentiation, and in cell–cell communication, in multicellular organisms [2].

A gap of over 1.5 billion years separated the appearance of single-celled eukaryotes and the evolution of the first multicellular eukaryotes [5–7]. While all organisms require signal transduction systems to respond to external cues, one critical requirement for multicellularity was the development of signaling mechanisms that enabled intercellular communication and coordination. Hunter and Cooper, in the first comprehensive review of tyrosine kinases (which appeared in 1985, only six years after the discovery of tyrosine phosphorylation), stated that "a clear prerequisite for a multicellular organism is a means of cell–cell signaling and the protein tyrosine kinase nature of many of the growth factor receptors may be pertinent" [8]. The requirement for intercellular signaling mechanisms raises the possibility that tyrosine kinases might be found in an organism close to the branch point with metazoans. This was confirmed in 2001 by King and Carroll in their studies of the unicellular choanoflagellate *Monosiga brevicollis*. These workers discovered the first TK-group kinase (a receptor tyrosine kinase designated MBRTK1) outside of the Metazoa [9]. A subsequent paper confirmed the existence of multiple active tyrosine kinases in this organism [10], leading to the suggestion that unicellular tyrosine kinases (and other key signaling molecules such as adhesion proteins) may have facilitated the evolution of multicellular animals [5].

Choanoflagellates are a group of protists that are believed to be the closest single-celled relatives to metazoans (Fig. 1) [11,12]. Many choanoflagellates can form colonies, suggesting that they may represent a transitional form between unicellular and multicellular organisms. Strikingly, genomic analyses have revealed that choanoflagellates contain numbers of tyrosine kinase genes that are comparable to (or even exceed) the numbers in the most complex multicellular animals [13]. In *M. brevicollis*, the number of tyrosine kinases has been estimated to be as high as 128, although estimates differ depending on the methodology used for identification [14,15]. Tyrosine kinases have also been identified in the choanoflagellates *Monosiga ovata* [16] and *Salpingoeca rosetta* [17]. Recent genome analyses indicate that tyrosine kinases are present in even more ancient opisthokonts (the eukaryotic supergroup that includes Fungi and Metazoa [7,18,19]). The filasterean *Capsaspora owczarzaki*, a member of a sister group to choanoflagellates and metazoans [20], has a rich and diverse tyrosine kinome (103 tyrosine kinase genes), and tyrosine kinases are present in *Ministeria vibrans*, a related

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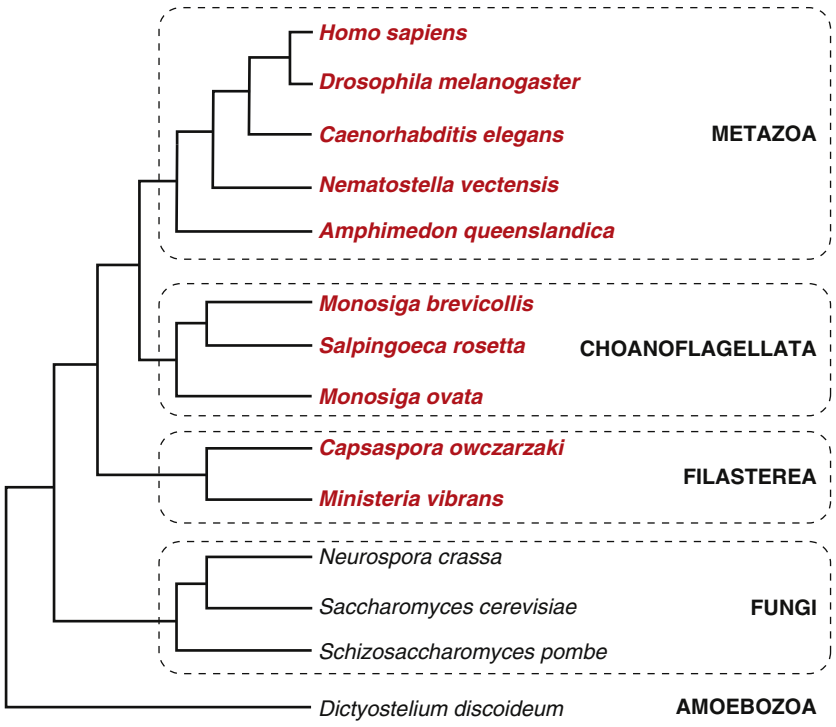


**Fig. 1.** Distribution of tyrosine kinases. The eukaryotic tree of life is shown schematically. Species containing eukaryotic-like tyrosine kinases are shown in red.

filasterean (H. Suga and I. Ruiz-Trillo, personal communication). Thus, the familiar tyrosine phosphorylation-based signaling system probably evolved before the divergence of *C. owczarzaki* from the branch including choanoflagellates and metazoans (Fig. 1). This innovation, which occurred ≈600 million years ago, may have been a critical event enabling the development of multicellular animals. Analysis of the genome of the sponge, *Amphimedon queenslandica*, one of the simplest and earliest branching metazoans, shows a dramatic expansion in the number of tyrosine kinases [21]. Remarkably, there are over 150 likely receptor tyrosine kinases in the sponge, including members of animal families such as the epidermal growth factor and Met receptors.

In present-day animal cells, the complete 'toolkit' of pTyr-based signaling consists of three components: (1) tyrosine kinases, which catalyze the modification; (2) protein tyrosine phosphatases (PTPs), which catalyze the opposing reaction (dephosphorylation of tyrosine residues); and (3) modular pTyr-binding domains (SH2, PTB). These three components have been designated "writers," "erasers," and "readers," respectively, by Wendell Lim and colleagues [14,22]. Regulatory circuits containing these three components play critical roles in the growth and differentiation of most metazoan cells. Based on the sequence of appearance of the three components, a model can be constructed for the evolution of pTyr signaling [22]. Genome analyses indicate that tyrosine phosphatases were the first to emerge in evolution. PTPs have been identified in several evolutionarily divergent protozoans that lack TK-group kinases, including *Leishmania* and *Trypanosoma* [23]. While the budding yeast *Saccharomyces cerevisiae* contains no tyrosine kinases and one primitive SH2 domain (which lacks the ability to bind p-Tyr), it has a few simple PTPs that are enzymatically active. These PTPs may have evolved to control phosphorylated tyrosine residues modified by yeast dual specificity kinases. (A caveat in considering *S. cerevisiae* as an example of a simple signaling system is that this organism lost a large number of genes since the radiation from the common ancestor with *S. pombe*) [24]. A more sophisticated p-Tyr signaling system is observed in organisms that contain "reader" domains in addition to PTPs. For example, the slime mold *D. discoideum* has a repertoire of 13 proteins containing SH2 domains but lacks metazoan-like tyrosine kinases [15,22]. The expansion of putative dual-specificity kinases in this organism, coupled with the lack of TK-group kinases, again suggests that SH2 domains initially evolved to control pathways involving dual-specificity catalytic domains.

The number and identity of signaling domains with which tyrosine kinases, PTPs, and SH2 domains are combined also serve as clues to the history of p-Tyr signaling [14,25]. In animal cells, p-Tyr signaling proteins tend to be large molecules composed of several modular signaling domains. This architecture is believed to reflect the importance of domain shuffling and recombination in the evolution of phosphotyrosine-based signaling. In contrast, the PTPs found in simple eukaryotes such as *Tetrahymena thermophila*, *S. cerevisiae*, and *D. discoideum* possess "stripped-down" architectures consisting of single-domain proteins or fusions with rhodanese domains [14,15,22]. Similarly, the 13 "reader" SH2 domains found in *D. discoideum* cluster into 5 basic architectures. The domain combinations in *Dictyostelium* are fairly limited, and do not display the kind of diversity observed in metazoan SH2 domain-containing proteins.

In contrast, with the emergence of the "writer" domain (i.e., the tyrosine kinase catalytic domain) closer to the advent of multicellular organisms, there was a flowering of domain combinations involving kinase, PTP, and SH2 domains. Choanoflagellates and metazoans have 30–50 PTP domains and up to 100–150 SH2 domains, about a 10-fold expansion as compared to *Saccharomyces* or *Dictyostelium* [14,15,22,25]. The number and variety of pairwise domain combinations are also greatly expanded in lineages containing tyrosine kinase domains. This reflects the increasing sophistication of pTyr-dependent regulatory circuits, in which the multidomain proteins can serve to integrate multiple signals, to bifurcate pathways, or to modulate the strength or duration of signaling [26,27]. Novel signaling systems based on tyrosine kinases, such as those observed in the nervous, vascular, and immune systems, arose at a later stage [28]. This explains the lack of kinases dedicated to these functions (e.g., immune cell tyrosine kinases such as Syk and ZAP-70) in choanoflagellates.

The domain architectures of the 128 tyrosine kinases in *M. brevicollis* are more varied than those seen in complex metazoans (Fig. 2) [15]. This reinforces the idea that tyrosine kinases evolved before the split between choanoflagellates and metazoans. The study of the *Monosiga*