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Research review paper

# Advances in kinome research of parasitic worms - implications for fundamental research and applied biotechnological outcomes

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ABSTRACT

Protein kinases are enzymes that play essential roles in the regulation of many cellular processes. Despite expansions in the fields of genomics, transcriptomics and bioinformatics, there is limited information on the kinase complements (kinomes) of most eukaryotic organisms, including parasitic worms that cause serious diseases of humans and animals. The biological uniqueness of these worms and the draft status of their genomes pose challenges for the identification and classification of protein kinases using established tools. In this article, we provide an account of kinase biology, the roles of kinases in diseases and their importance as drug targets, and drug discovery efforts in key socioeconomically important parasitic worms. In this context, we summarise methods and resources commonly used for the curation, identification, classification and functional annotation of protein kinase sequences from draft genomes; review recent advances made in the characterisation of the worm kinomes; and discuss the implications of these advances for investigating kinase signalling and developing small-molecule inhibitors as new anti-parasitic drugs.

## 1. Introduction - history and significance of kinase research

Knowledge of cell signalling is crucial to understanding eukaryotic organisms. In 1955, the principle of reversible protein phosphorylation involving phosphorylating (kinases) and de-phosphorylating (phosphatases) enzymes was discovered (Fischer and Krebs, 1955; Sutherland Jr and Wosilait, 1955). Then, the discovery that all cells contain deoxyribonucleic acid (DNA), which holds the key to producing messenger RNA (mRNA) and the synthesis of proteins that assume essential structural and enzymatic functions in all cells, led to the formulation of the “Sequence Hypothesis” and the “Central Dogma” of molecular biology (Crick, 1958, 1970). These early discoveries, followed by advances in the ability to determine nucleotide and amino acid sequences (Edman and Begg, 1967; Jay et al., 1974; Maxam and Gilbert, 1977; Padmanabhan et al., 1974; Sanger and Coulson, 1975; Sanger et al., 1977; Wu, 1972), enabled studies of single genes, transcripts and proteins, including protein kinases (Fig. 1). Progress in sequencing technologies allowed kinase genes - mainly from mammalian cell lines, vinegar fly (*Drosophila melanogaster*) and yeast (*Saccharomyces cerevisiae*) - to be characterised, without having to purify kinases and test their activity (Hanks, 1987; Hunter, 1987). It also enabled the comparison of inferred kinase sequences, the definition of conserved residues, domains and subdomains, and the construction of the first phylogeny of protein kinases (Hanks et al., 1988).

Functional studies, first in yeast, revealed an involvement of kinases in cell cycle progression and sexual differentiation, which led to the discovery of roles of cyclin-dependent kinases and mitogen-activated protein kinases (MAPKs) in these processes, respectively (Brizuela et al., 1987; Draetta et al., 1987; Lee and Nurse, 1987; Reed et al., 1985; Simanis and Nurse, 1986). Other investigations explored signalling processes in multicellular model organisms, such as *D. melanogaster* (see Duffy and Perrimon, 1996; Perrimon, 1994) and the free-living nematode *Caenorhabditis elegans* (see Duggan and Chalfie, 1995; Eisenmann and Kim, 1994; Sternberg and Horvitz, 1991).

Solving the three-dimensional crystal structure of the catalytic subunit of a kinase - the cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA) (Knighton et al., 1991; Zheng et al., 1993) provided first, crucial insight into kinase sub-structures and their roles in catalysing protein phosphorylation. This work also laid the foundation for intensive research on small molecules that inhibit mutated/deregulated protein kinases, an avenue that had been proposed in earlier studies showing roles of kinase oncogenes in the growth of viral tumours in birds (Brugge and Erikson, 1977; Hunter and Sefton, 1980; Martin, 1970) and cancers of humans (Heisterkamp et al., 1983; Morange, 1993; Shtivelman et al., 1985; Varmus, 1985) (Fig. 1).

Further advances in sequencing technologies in the 1990s (Burke

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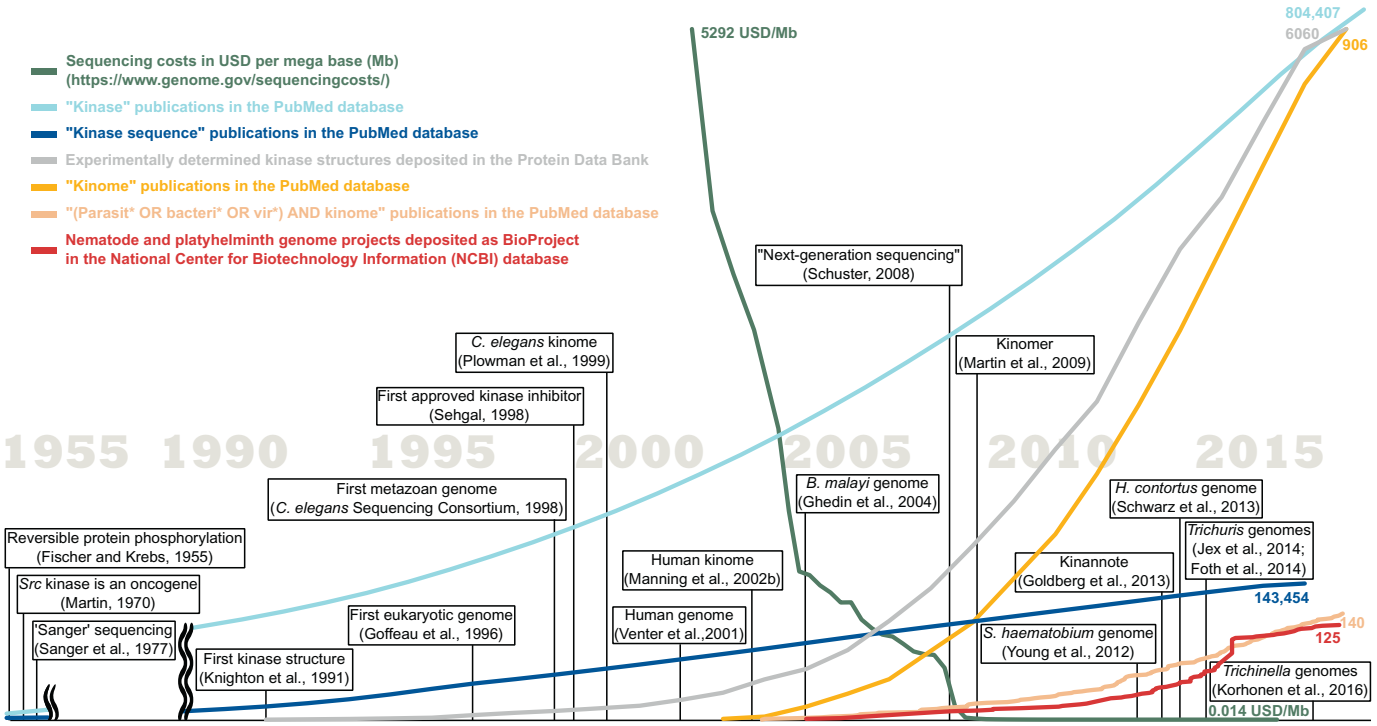


**Fig. 1.** Timeline showing advances in genome and transcriptome sequencing, protein kinase research, as well as genome and kinome research of parasitic helminths. All values represented as lines have been min-max normalised for display purposes and some (light blue and blue; orange, beige and brown) have been normalised together to allow for visual comparisons. The latest values for individual database searches are given at the end of each line. Black, wavy lines represent a break in the timeline.

**Table 1**
The kinomes of 15 representative organisms in KinBase - kinase group classifications.

| Name | Description | Totals | AGC group | CAMK group | CK1 group | CMGC group | RGC group | STE group | TK group | TKL group | "Other" group | aPKs/ PKLs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Homo sapiens* | Human | 538 | 63 | 74 | 12 | 64 | 5 | 47 | 90 | 43 | 81 | 59 |
| *Mus musculus* | Mouse | 557 | 60 | 96 | 11 | 62 | 7 | 47 | 90 | 43 | 83 | 58 |
| *Strongylocentrotus purpuratus* | Sea urchin | 354 | 29 | 48 | 6 | 35 | 8 | 21 | 54 | 38 | 94 | 21 |
| *Drosophila melanogaster* | Vinegar fly | 237 | 30 | 32 | 10 | 34 | 6 | 18 | 31 | 17 | 44 | 15 |
| *Caenorhabditis elegans* | Nematode worm | 438 | 29 | 42 | 83 | 48 | 27 | 24 | 85 | 15 | 67 | 18 |
| *Amphimedon queenslandica* | Sponge | 703 | 27 | 35 | 5 | 42 | 3 | 26 | 222 | 248 | 70 | 25 |
| *Monosiga brevicollis* | Unicellular choanoflagellate | 114* | 0 | 0 | 0 | 0 | 0 | 0 | 109 | 0 | 0 | 5 |
| *Saccharomyces cerevisiae* | Baker's yeast | 132 | 17 | 22 | 4 | 23 | 0 | 14 | 0 | 0 | 38 | 14 |
| *Coprinopsis cinerea* | Mushroom | 374 | 23 | 18 | 5 | 31 | 0 | 18 | 0 | 18 | 182 | 79 |
| *Dictyostelium discoideum* | Amoebozoan slime mold | 294 | 21 | 21 | 3 | 30 | 0 | 45 | 0 | 67 | 68 | 39 |
| *Tetrahymena thermophila* | Free-living ciliate | 1114 | 60 | 63 | 20 | 65 | 0 | 19 | 0 | 8 | 760 | 119 |
| *Gardia lamblia* | Excavate protozoan | 283 | 7 | 8 | 1 | 24 | 0 | 8 | 0 | 0 | 226 | 9 |
| *Leishmania major* | Kinetoplastid protozoan | 223 | 11 | 23 | 7 | 50 | 0 | 43 | 0 | 0 | 61 | 28 |
| *Trichomonas vaginalis* | Excavate protozoan | 1076 | 90 | 442 | 71 | 136 | 0 | 27 | 0 | 121 | 123 | 66 |
| *Selaginella moellendorffii* | Lycophyte (plant) | 1013 | 33 | 139 | 9 | 93 | 0 | 36 | 0 | 576 | 77 | 50 |

* The number of sequences reported in KinBase is 261, including proteins labelled "TK-associated" and "PTP". These sequences do not contain a kinase catalytic domain and therefore are not listed here, except for five sequences (listed as PKLs)

et al., 1987; Kim et al., 1996; O'Connor et al., 1989; Shizuya et al., 1992) made it possible to characterise transcriptomes (Adams et al., 1995; Korenberg et al., 1995) and genomes of key eukaryotic organisms (Gibbs, 1995; Little, 1995). The draft genomes of *S. cerevisiae* (see Goffeau et al., 1996), *C. elegans* (see *C. elegans* Sequencing Consortium, 1998), *D. melanogaster* (see Adams et al., 2000), mouse (Mouse Genome Sequencing Consortium et al., 2002) and human (Venter et al., 2001) enabled, for the first time, protein kinase complements (kinomes) of these organisms to be defined (Caenepeel et al., 2004; Hunter and Plowman, 1997; Manning et al., 2002a, 2002b; Morrison et al., 2000; Plowman et al., 1999) (Table 1). These studies provided insights into kinase evolution (Kannan et al., 2007b; Manning et al., 2002a) and enabled functional studies of entire kinomes, mainly in the nematode *C. elegans* (see Lehmann et al., 2013; Maeda et al., 2001; Reinke et al., 2000). In ensuing years, there was an increased effort to sequence other worms (helminths), with a focus on parasitic species of socioeconomic importance. The first draft genome of a parasitic helminth was that of the filarial nematode *Brugia malayi* (see Blaxter et al., 2002; Ghedin et al., 2004, 2007), which allowed for the characterisation of its kinome. Comparisons with *C. elegans* and *C. briggsae* revealed