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# 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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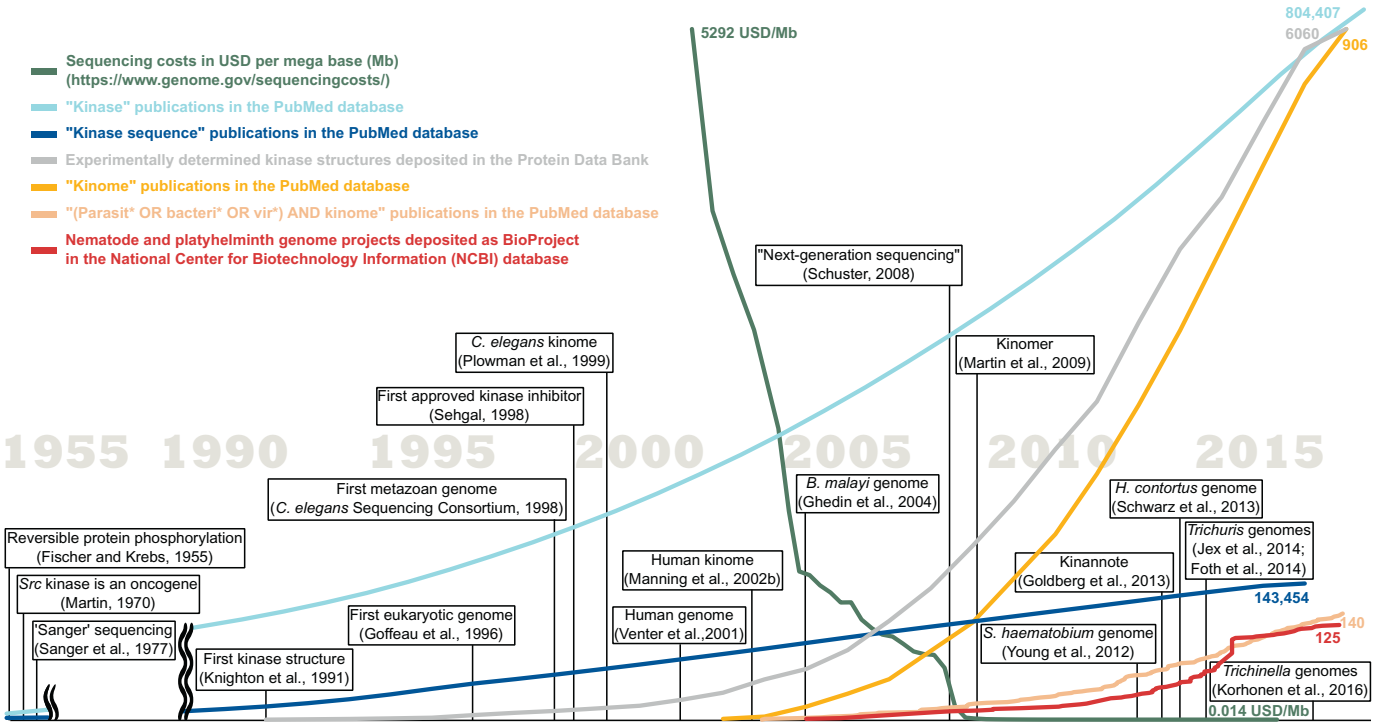


**Fig. 1.** Timeline showing advances in genome and transcriptome sequencing, protein kinase research, as well as genome and kinome research of parasitic helminths. All values represented as lines have been min-max normalised for display purposes and some (light blue and blue; orange, beige and brown) 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

considerable differences in kinome composition between *B. malayi* and these free-living nematodes.

The advent of advanced, short-read ("next-generation") sequencing (NGS) technologies, such as the Illumina Solexa platform (Mardis, 2008a, 2008b; Metzker, 2005; Schuster, 2008), marked another milestone and allowed many other genomes to be sequenced at a fraction of the cost spent on the first eukaryotic genome projects, and in a relatively short time frame. This progress led to a massive expansion in the number of genome projects (Fig. 1), resulting in numerous draft genomes for parasitic worms (Berriman et al., 2009; Foth et al., 2014; Jex et al., 2011, 2014; Korhonen et al., 2016; Laing et al., 2013; Mitreva et al., 2011; Protasio et al., 2012; *Schistosoma japonicum* Genome Sequencing and Functional Analysis Consortium, 2009; Schwarz et al., 2013, 2015; Tang et al., 2014; Young et al., 2012; Zhu et al., 2015). Genomic and transcriptomic data sets for parasitic helminths (cf. WormBase ParaSite; Howe et al., 2017) now provide an unprecedented resource for the exploration of kinomes and kinase signalling pathways (Bethony et al., 2006; Brindley and Hotez, 2013; Brindley et al., 2009; Hotez et al., 2009; Hotez and Kamath, 2009). Given that protein kinases have been recognised as critical in the pathogenesis of some diseases (Brugge and Erikson, 1977; Heisterkamp et al., 1983; Hunter and Sefton, 1980; Martin, 1970; Morange, 1993; Shtivelman et al., 1985; Varmus, 1985) and as drug targets to combat non-infectious (Blume-Jensen and Hunter, 2001; Cohen, 2001, 2002; Cohen and Alessi, 2013) and parasitic diseases (Beckmann et al., 2012; Lucet et al., 2012; Morel et al., 2014), investigating kinases in parasitic worms might hold a key to designing new interventions against parasitic helminths.

This article reviews salient aspects of kinase biology and drug discovery; critically appraises methods and resources commonly used for the characterisation of protein kinase sequences from draft genomes; reports on recent technological advances in the characterisation of worm kinomes; and discusses the implications of understanding kinase biology and signalling processes in worms, particularly in relation to biotechnological advances.

## 2. Protein kinase complements, their evolution and classification

Protein kinases are enzymes (transferases) that phosphorylate a substrate *via* the transfer of a phosphoryl group from an energy-rich molecule (adenosine triphosphate; ATP). The phosphorylation of the substrate induces a structural or biochemical modification that leads to changes in its activity, reactivity and/or conformation. Phosphorylation can take place at different sites of an amino acid residue; for serine/threonine (Graves and Krebs, 1999; Krebs, 1993) and tyrosine (Hubbard and Till, 2000; Schlessinger and Ullrich, 1992), the hydroxyl group is phosphorylated, whereas for histidine, arginine and lysine, phosphorylation occurs on a nitrogen (Matthews, 1995). Additionally, some kinases phosphorylate themselves (autophosphorylation) or are phosphorylated by other kinases. Based on the residues that they recognise and phosphorylate, most kinases belong to one of two main superfamilies: the serine/threonine kinases (STKs) and the tyrosine kinases (TKs). Although most kinases specifically recognise and phosphorylate only one type of residue, others (termed "dual-specificity kinases") act on both serine/threonine and tyrosine residues (Hunter et al., 1992; Stern et al., 1991).

Kinases represent one of the largest classes of proteins in most eukaryotic genomes, representing ~2–4% of protein-coding genes (Champion et al., 2004; Hanks, 2003). They are ubiquitously present in eukaryotes and are involved in most signal transduction pathways, regulating a wide range of cellular functions, such as cell growth, proliferation, transcriptional regulation, apoptosis and the cellular sub-localisation of proteins (Cohen, 2000; Manning, 2005). Given their importance in these processes, the structure and function of protein kinases have been investigated for decades (reviewed by Endicott et al., 2012; Johnson and Hunter, 2005; Knight et al., 2013; Schlessinger, 2014), mainly in tractable model organisms including *S. cerevisiae* (see Bharucha et al., 2008; Sharifpoor et al., 2012) and *C. elegans* (see Kamath and Ahringer, 2003; Lehmann et al., 2013; Maeda et al., 2001; Plowman et al., 1999; Sonnichsen et al., 2005; Sugimoto, 2004) and, recently, in eukaryotic pathogens, such as malaria parasites (*Plasmodium* spp.) (Carvalho et al., 2016) and blood flukes (schistosomes; *Schistosoma* spp.) (Beckmann et al., 2012; Morel et al., 2014). These studies have greatly advanced the understanding of the molecular biology and cellular signalling mechanisms of eukaryotes. The increasing availability of kinase sequences for single-celled and multicellular eukaryotes has enabled the analysis of their phylogenetic relationships and provided first insights into kinase evolution and function (Goldberg et al., 2006; Manning, 2005; Manning et al., 2002a). Such studies revealed lineage-specific expansions and contractions, and facilitated the classification of kinases into groups that are recognised as functionally and structurally conserved throughout evolution (Hanks, 2003; Hanks and Hunter, 1995; Manning, 2005; Manning et al., 2002a). This classification is based on sequence similarity within the catalytic kinase domain, the presence of additional domains, known biological functions and levels of sequence conservation across divergent taxa. The present protein kinase classification scheme is based on the original scheme developed by Hanks and Hunter (Hanks and Hunter, 1995; Hanks et al., 1988) and was subsequently refined (Hanks, 2003; Manning et al., 2002a, 2002b). The currently accepted "Standard Kinase Classification Scheme" (http://kinase.com/wiki/index.php/Standard_Kinase_Classification_Scheme) is subject to change, as new kinases are discovered or existing kinases are reclassified into new families and/or subfamilies based on their structure and/or function. Eukaryotic protein kinases (ePKs) all share a conserved catalytic domain and are divided into nine groups (Table 1):

1. The AGC group contains kinases that are modulated by cyclic nucleotides, phospholipids or calcium. This group is represented mainly by the protein kinase families A, G and C (PKA, PKG, PKC), Akt, 3-phosphoinositide-dependent kinase 1 (PDK1), dystrophia myotonica protein kinase (DMPK), microtubule-associated Ser/Thr kinase (MAST), ribosomal S6 kinase (RSK), nuclear DbF2-related kinase and G protein-coupled receptor kinases (GRKs) (Pearce et al., 2010).
2. The CAMK group consists of two calmodulin/calcium-regulated kinase families (CAMK1 and CAMK2) and several families of kinases that are not regulated by calcium, which include CAMKL (CAMK-like) kinases and brain-selective kinases (BRSKs) (Soderling, 1999; Swulius and Waxham, 2008).
3. The CMGC group is usually one of the largest groups of kinases, consisting of four families (cyclin-dependent kinases (CDKs), mitogen-activated protein kinases (MAPKs), glycogen synthase kinase 3 (GSK) and cell division cycle (CDC)-like kinases (CLKs)), members of which play diverse roles in cell cycle control, transcriptional regulation, splicing and other processes (Krishna and Narang, 2008; Varjosalo et al., 2013).
4. The CK1 group contains a small number of representatives for most metazoans but is expanded in many nematode species including *C. elegans* and *C. briggsae* (see Manning, 2005). This group is subdivided into the families casein kinase 1 (CK1), venus kinase receptors (VKRs) (Vanderstraete et al., 2013), Tau tubulin kinases (TTBKs) and TTBK-like kinases (TTBKLs) (Ikezu and Ikezu, 2014).
5. The group of receptor guanylate cyclases (RGCs) is a relatively small group in most organisms, representing sequences that contain an active guanylate cyclase domain and a catalytically inactive kinase domain that appears to have a regulatory function (Garbers, 1990; Jaleel et al., 2006; Singh et al., 1988). Similar to CK1, this group is also substantially expanded in nematodes such as *C. elegans*, *C. briggsae* and *Ancylostoma ceylanicum* (see Manning, 2005; Zaru et al., 2017).
6. The STE group contains homologs of proteins encoded by the yeast *sterile* (*ste*) genes (STE7, STE11 and STE20) that form the MAPK

cascade, transducing signals from the surface of the cell to the nucleus, thus regulating gene expression in response to extracellular stimuli (Chang and Karin, 2001).

7. The group of tyrosine kinases (TKs) represents kinases that phosphorylate its substrate on tyrosine residues, as opposed to most kinases from the other groups that phosphorylate either serine or threonine residues. TKs can be further subdivided into receptor TKs (RTKs) and non-transmembrane (i.e. cytoplasmic) TKs (CTKs), and play vital roles in cell proliferation, differentiation, subcellular localisation and metabolism (Hubbard and Till, 2000; Schlessinger and Ullrich, 1992).
8. Tyrosine kinase-like kinases (TKLs) are similar in sequence to tyrosine kinases but usually phosphorylate substrates on serine/threonine residues. This group includes families that form part of the MAPK cascade (MAP3Ks), receptor kinase families as well as kinases involved in immunity and cytoskeletal processes (Goldberg et al., 2006). Several families in this group are expanded in plants and *Dictyostelium discoideum*, suggesting that they are of ancient origin (Goldberg et al., 2006; Zulawski et al., 2014).
9. The "Other" group is a diverse group of kinase families and single kinases that are ePKs but share limited similarity with any of the eight other ePK groups (Hanks, 2003; Manning et al., 2002a). In humans, the largest family in this group is the "never-in-mitosis A" (NIMA)-related kinase (NEK) family, members of which play important roles in cell cycle regulation (Fry et al., 2012). Other examples include *unc-51*-like autophagy-activating kinases (ULKs) (Nazio and Cecconi, 2017) and polo-like kinases (PLKs), the latter of which play roles in the regulation of the cell cycle and in stress response mechanisms (Strebhardt, 2010).

In addition to these nine recognised groups of ePKs, many other kinases have the same protein kinase-like (PKL) fold and catalytic mechanism, despite sharing limited sequence similarity. Thus, divergent families within the PKL superfamily, including Alpha, ABC1 (ABC1 domain-containing), PIKK (phosphatidyl inositol 3' kinase-related kinases) and RIO (right open reading frame), are termed 'atypical' kinases (aPKs) or simply PKLs (Kannan et al., 2007b; Scheeff and Bourne, 2005). Taken together, the nine ePK groups, the aPKs and other members of the PKL superfamily are currently divided into 240 families and 218 subfamilies (http://kinase.com/kinbase/).

## 3. Structural and biochemical aspects of kinase function

Despite their sequence and functional divergences, most eukaryotic kinases have a conserved protein kinase catalytic domain consisting of twelve sequence subdomains (Fig. 2A) with several highly conserved residues that are essential for catalytic activity (Hanks, 2003). The first solved crystal structure of PKA and genome-wide comparisons of kinases provided insights into how these conserved residues interact with ATP and protein substrates and into the role(s) that they play in the three-dimensional conformation of kinases required for catalytic activity (Knighton et al., 1991; Zheng et al., 1993). These studies revealed that the catalytic domain of kinases assume a bilobal structure (Fig. 2B). The smaller, amino- (N-) terminal lobe includes the first four of the twelve subdomains and functions mainly in ATP binding, but can also play roles in kinase activation by providing a binding site for other proteins, such as cyclins (Morgan, 1997; Varjosalo et al., 2013). Subdomain I, also known as "glycine-rich loop", has a conserved Gly-x-Gly-x-x-Gly motif and represents the most mobile part of the protein kinase, assuming an 'open' or a 'closed' conformation. This loop consists of two β-strands; in the closed conformation, it anchors the α- and β- (i.e. the non-transferrable) phosphates and positions the γ-phosphate of ATP for its transfer to the protein substrate (Hanks and Hunter, 1995; Johnson et al., 2001). Subdomain II is represented by β-strand 3 in the small lobe and contains a lysine residue that is critical for catalytic function. This residue plays an important role in orienting the ATP molecule by

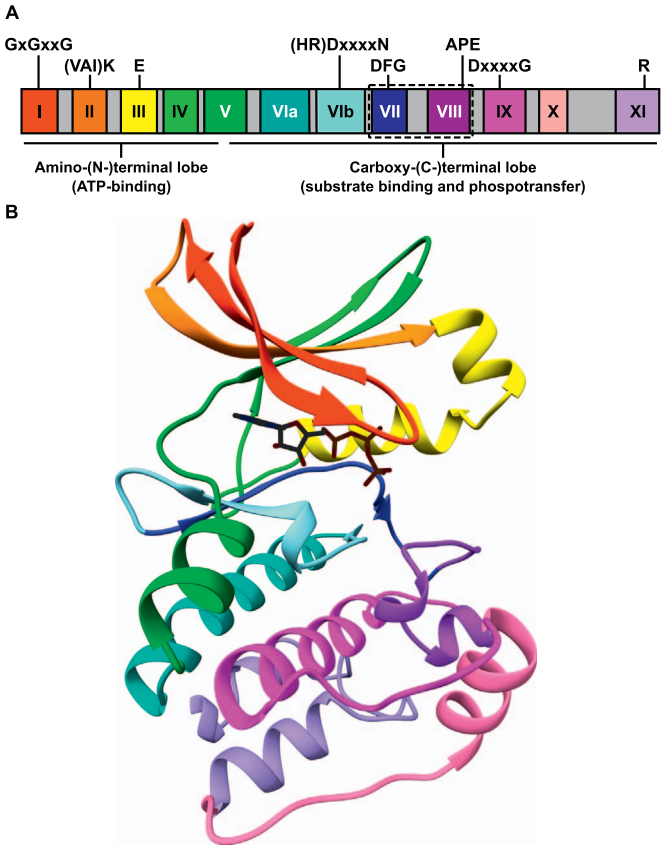


**Fig. 2.** Salient sequence and structural features of the protein kinase catalytic domain. (A) The twelve conserved subdomains (colour-coded) and important functional residues are shown (adapted from Hanks, 2003). Grey areas represent less conserved sequence regions. The dashed box represents the activation segment. (B) Representative crystal structure of a protein kinase catalytic domain in the active ('DFG-in') conformation and a bound adenosine triphosphate (ATP) in a black stick representation (Protein Data Bank identifier: 1ATP; adapted from Zheng et al., 1993). Subdomains are colour-coded in concordance with (A).

interacting with its α- and β-phosphates, and is often part of a Val-Ala-Ile-Lys (VAIK) motif (Hanks and Hunter, 1995; Pearce et al., 2010). Strand 3 is followed by a small helix ("B-helix"), which can be variable in length and sequence or is missing from some protein kinases altogether, and by the large "C-helix" (subdomain III) that contains a conserved glutamic acid residue. This residue is centrally located in the C-helix and plays a stabilising role in the interactions between the lysine in subdomain II and the α- and β-phosphates of ATP. This subdomain is the only subdomain that contains helical elements in the N-lobe of the kinase core. The hydrophobic β-strand 4 constitutes subdomain IV that does not contain conserved residues and has no known direct role in the recognition of a kinase substrate or catalytic function.

The last β-strand of the N-lobe is linked to the first α-helix of the larger C-lobe *via* subdomain V ("hinge region"). The C-lobe is comprised mostly of α-helices and harbours structural elements and residues important for peptide binding and catalytic processes. This lobe represents the more stable part of the kinase structure, compared with the relatively flexible N-lobe. Subdomain VIa ("E-helix") represents a very hydrophobic part of the kinase structure and is of structural importance, but does not directly play a role in either ATP or substrate binding. Subdomain VIb consists of the two β-strands 6 and 7, and has a conserved His-Arg-Asp-x-x-x-x-Asn (HRDxxxxN) motif. This subdomain is also known as the "catalytic loop", because the aspartic acid residue in this loop acts as a catalytic base in the phosphotransfer process, and the asparagine facilitates the positioning of a divalent $Mg^{2+}$ cation which interacts with the oxygens of the α- and γ-phosphates of the ATP

(Adams, 2001; Zheng et al., 1993). Subdomain VII represents the "Mg-binding loop" and the "activation loop" (or "T-loop"). The Mg-binding loop contains an Asp-Phe-Gly (DFG) motif, in which the aspartic acid assumes a critical role in the recognition of a second $Mg^{2+}$ ion that bridges the β- and γ-phosphates of ATP (Adams, 2001; Taylor and Kornev, 2011; Zheng et al., 1993). The activation loop requires phosphorylation at a serine/threonine or tyrosine residue, which interacts with conserved residues in both the activation loop and the catalytic loop (typically the HRD-arginine) to stabilise a catalytically active kinase conformation (Taylor and Kornev, 2011). The next subdomain (VIII; "P + 1 loop") is a stable structural element that contains a conserved Ala-Pro-Glu (APE) motif, which is important for docking of the peptide substrate and for C-lobe stability (Hanks and Hunter, 1995; Nolen et al., 2004). Together, the Mg-binding loop, activation loop and P + 1 loop constitute the "activation segment", which, upon phosphorylation of a residue within the activation loop, assumes a relatively well-conserved, active ('DFG-in') conformation, forming a hydrophobic, regulatory spine (R-spine) that links the two kinase lobes (Kornev et al., 2006; Taylor and Kornev, 2011). In contrast, the R-spine is disassembled in inactive kinases and the activation segment can assume a variety of different configurations including a DFG-in-like arrangement (Taylor and Kornev, 2011). One inactive conformation (termed 'DFG-out') common to many kinases is caused by a mechanism in which the incorrect positioning of the DFG motif sterically blocks ATP binding and leads to breakage of the R-spine (Taylor and Kornev, 2011).

Subdomain IX ("F-helix") is a hydrophobic helix that spans the centre of the C-lobe and is structurally important (Kornev and Taylor, 2010; Kornev et al., 2008). Subdomain X ("G- helix") is less conserved among different protein kinases compared with other subdomains, and represents a recognition- and docking-site for protein substrates (Dar et al., 2005; Kornev and Taylor, 2010). The last subdomain (XI) of the C-lobe is the "H-helix", which has a conserved arginine residue that interacts with the conserved glutamic acid in the APE motif of subdomain VIII, thus stabilising the C-lobe (Hanks and Hunter, 1995).

In addition to these conserved subdomains and residues in the catalytic core of kinases, these enzymes often have non-catalytic accessory domains that are fused N- and/or C-terminally to the catalytic subunit (for an extended list of known accessory domains in kinases, see Manning et al., 2002b). These domains form an important part of many kinase signalling cascades, assuming crucial roles in protein-protein interactions, kinase activation and dimerisation (Huse and Kuriyan, 2002; Kannan et al., 2007a; Langeberg and Scott, 2015). In some cases, regulatory subunits are encoded by a separate gene and form a functional complex with the catalytic subunit (Berman et al., 2005).

Although most of the structural properties of protein kinases were initially derived from the crystal structure of PKA, the structures of many other kinases were solved shortly thereafter (for early reviews, see Bossemeyer, 1995; Hubbard and Till, 2000), which provided new insights into mechanisms of kinase activation and phosphorylation, revealed variation in activation loops and regulatory mechanisms among kinases and helped better understand which structural features render a kinase catalytically inactive (i.e. a pseudokinase) (Langeberg and Scott, 2015; Nolen et al., 2004; Taylor and Kornev, 2011). As of November 2017, there were more than 5800 solved crystal structures of protein kinases in the Protein Data Bank (PDB; http://www.rcsb.org/pdb/), including 3648 protein-serine/threonine kinases (Enzyme Commission (EC) number: 2.7.11), 1723 protein-tyrosine kinases (EC number: 2.7.10), 275 protein-histidine kinases (EC number: 2.7.13) and 182 dual-specificity kinases (EC number: 2.7.12).

## 4. Kinases as drug targets

An increased focus on structural investigations of protein kinases has been driven mainly by the fact that mutated or otherwise deregulated kinases are involved in the onset of diseases such as cancers (Cohen, 2001). For a comprehensive review of disease-associated kinase mutations, the reader is referred to a recent publication by Lahiry et al. (2010).

A better understanding of protein kinases and their structures has enabled new strategies of targeting them with small molecule compounds. This task was previously deemed challenging, if not impossible, given the high intracellular concentration of ATP with which a small molecule inhibitor would have to compete and the challenges associated with the highly selective targeting of a structurally conserved site for ATP recognition (Cohen, 2002).

Despite these challenges, between 1999 and 2001, two compounds shown to target and selectively inhibit protein kinases were approved by the US Food and Drug Administration (FDA): rapamycin (sirolimus; Sehgal, 1998) and gleevec (imatinib; Druker et al., 2001). These discoveries resulted in an increased interest in protein kinases as drug targets, rapidly making them the second-most targeted proteins after G-protein-coupled receptors (Cohen, 2002). In the last decade, many kinase inhibitors have been investigated as drugs against a range of illnesses including inflammatory and autoimmune disorders, Parkinson's disease, hypertension and different types of human cancers (Cohen and Alessi, 2013). As of July 2015, there were 28 FDA-approved drugs that target protein kinases (for a review of human kinase targets and associated inhibitors, see Wu et al., 2016), predominantly indicated for use as anti-cancer agents (Eglen and Reisine, 2009, 2011; Wu et al., 2015). Most kinases ($n = 24$) targeted by these drugs are tyrosine kinases (TK), and only two targets belong to each of the three groups CMGC, STE and TKL (Wu et al., 2016). An analysis of ~19,000 inhibitors (Hu et al., 2015) showed that, although members of eight major kinase groups have been investigated as drug targets, to date, kinases representing less than half of the human kinome have been targeted using small molecules (Fedorov et al., 2010).

Most of these compounds are so-called Type I inhibitors - ATP-competitive molecules that bind to the active ('DFG-in') conformation of kinases (Zhang et al., 2009). In contrast, Type II inhibitors bind to and stabilise the inactive ('DFG-out') kinase conformation (Liu and Gray, 2006; Zhao et al., 2014). However, human tumour cells can become resistant to such inhibitors (the most prominent example being imatinib; Krishnamurty and Maly, 2010) if a residue in the hinge region of a kinase (the 'gatekeeper' residue) is mutated (Gorre et al., 2001; Liu et al., 1998), thus stabilising a conserved hydrophobic spine (Kornev et al., 2006, 2008), activating the kinase and sterically blocking access to the inhibitor binding site (Azam et al., 2008; Daub et al., 2004).

To overcome resistance and/or to achieve higher target selectivity, compounds were developed that do not bind to the ATP-binding site, but rather target an adjacent pocket (Type III inhibitor), a remote site (Type IV inhibitor), or bivalently bind to both the ATP binding pocket and the substrate recognition site (Type V inhibitor) (Cox et al., 2011; Gavrin and Saiah, 2013; Müller et al., 2015). Although the latter three inhibitor types have the advantage of usually being more selective, the promiscuity associated with ATP-competitive inhibitors has been successfully exploited to target multiple kinase targets at the same time with just one drug ("targeted polypharmacology"; Achenbach et al., 2011; Bilanges et al., 2008; Hopkins et al., 2006; Metz and Hajduk, 2010).

## 5. Parasitic helminths - socioeconomic importance and biology

The fact that kinases can be relatively selectively targeted by small molecule compounds and that they play important roles in most biological signalling processes and in disease, has spawned studies of these enzymes as drug targets in eukaryotic pathogens (Lucet et al., 2012), including parasitic worms (Beckmann et al., 2012; Morel et al., 2014). Parasites often occupy unique biological niches within their host (Despommier, 1993; Garnick, 1992) and have ways of evading or avoiding host immune detection or attack (Maizels and McSorley, 2016; Maizels et al., 2009; McSorley et al., 2013), which often requires specialised signalling mechanisms (Gilabert et al., 2016; Lok, 2016).

**Table 2**
Kinome sizes estimated from published genomes and transcriptomes of parasitic helminths, and numbers of predicted targets for anthelminthic drugs.

| Species name | Estimated kinome size | Number of predicted drug targets | Reference |
|---|---|---|---|
| *Ancylostoma ceylanicum* | ~365 | 0 | (Schwarz et al., 2015) |
| *Angiostrongylus cantonensis* | 361 | n.r. | (Yong et al., 2015) |
| *Ascaris suum* | 609 | 17 | (Jex et al., 2011) |
| | 364 | 7 | (Desjardins et al., 2013) |
| *Brugia malayi* | 215 | n.r. | (Ghedin et al., 2007) |
| | 282 | 6 | (Desjardins et al., 2013) |
| | 465 | TTBKL and FER kinases | (Bennuru et al., 2016) |
| *Clonorchis sinensis* | 692 | n.r. | (Wang et al., 2011) |
| | n.r. | n.r. | (Huang et al., 2013) |
| *Dirofilaria immitis* | 283 | TTBKL and FER kinases | (Bennuru et al., 2016) |
| *Fasciola hepatica* | ~307 | n.r. | (McNulty et al., 2017) |
| *Haemonchus contortus* | 845 | 27 | (Schwarz et al., 2013) |
| | n.r. | 0 | (Laing et al., 2013) |
| *Loa loa* | 310 | 7 | (Desjardins et al., 2013) |
| | 306 | TTBKL and FER kinases | (Bennuru et al., 2016) |
| *Meloidogyne hapla* | 234 | 6 | (Desjardins et al., 2013) |
| *Necator americanus* | 274 | 59 (32 with associated drugs) | (Tang et al., 2014) |
| *Onchocerca ochengi* | 282 | TTBKL and FER kinases | (Bennuru et al., 2016) |
| *Onchocerca volvulus* | 318 | TTBKL and FER kinases | (Bennuru et al., 2016) |
| *Opisthorchis viverrini* | 262 | n.r. | (Young et al., 2014) |
| *Pristionchus pacificus* | 346 | 6 | (Desjardins et al., 2013) |
| *Schistosoma haematobium* | 261 | 0 | (Young et al., 2012) |
| *Schistosoma japonicum* | n.r. | n.r. | (*Schistosoma japonicum* Genome Sequencing and Functional Analysis Consortium, 2009) |
| *Schistosoma mansoni* | 249 | 1 | (Berriman et al., 2009) |
| | 252 (ePKs only) | 15 | (Andrade et al., 2011) |
| | n.r. | 18 | (Caffrey et al., 2009) |
| *Toxocara canis* | 458 | 57 | (Zhu et al., 2015) |
| *Trichinella spiralis* | 233 | 7 | (Desjardins et al., 2013) |
| *Trichuris suis* | 232 | n.r. | (Jex et al., 2014) |
| *Trichuris trichiura* | n.r. | 2 | (Foth et al., 2014) |
| *Wuchereria bancrofti* | 304 | 10 | (Desjardins et al., 2013) |
| | 230 | TTBKL and FER kinases | (Bennuru et al., 2016) |

n.r., not reported

Although there is a limited understanding of the biochemical signalling pathways governing complex biological traits, the life cycles of some parasitic worms are relatively well understood, and often involve one or more hosts required for development and reproduction. The following sections provide an account of the life cycles and socioeconomic importance of key parasitic helminths. The focus is on representative species of flatworms and roundworms, for which progress has recently been made in the curation and characterisation of kinases. For further information on the biology of other important worms, the reader is referred to Table 2.

### 5.1. Parasitic flatworms (*Schistosoma* species)

Schistosomiasis is a neglected tropical disease (NTD) caused by blood flukes (schistosomes) of the genus *Schistosoma* (phylum Platyhelminthes; class Trematoda). At least 230 million people are infected with schistosomes, and almost 700 million are at risk of contracting an infection (Colley et al., 2014; World Health Organization, 2012a). Schistosomiasis is responsible for more than three million of a total of 26 million disability-adjusted life years (DALYs) attributable to all NTDs (Fenwick, 2012; King et al., 2005; Murray et al., 2012). Two species, *Schistosoma haematobium* and *S. mansoni*, are the cause of more than 160 million infections in sub-Saharan Africa, with *S. mansoni* causing greater mortality and being accountable for about two thirds of all schistosome infections in this geographic region (van der Werf et al., 2003; World Health Organization, 2012a). *Schistosoma mansoni* is also found in Latin America, whereas another species, *S. japonicum*, is present mainly in China and South-East Asia (Colley et al., 2014).

Schistosomes have complex, indirect life cycles that require a mollusc as an intermediate host (Roberts and Janovy Jr., 2009). Their generalised life cycle has numerous phases: a ciliated larva, called a miracidium, develops inside an egg, hatches in water and penetrates a freshwater snail. Once inside this host, it sheds its ciliated ectoderm and develops to a sporocyst. This developmental stage undergoes asexual reproduction, giving rise to daughter generations of sporocysts. Embryos within the sporocysts then develop to cercariae that emerge from the mollusc and penetrate the skin of a definitive host (human). Following penetration, the cercariae shed their tails and are disseminated *via* the blood stream. Juvenile schistosomules then mature into dioecious adults that mate, migrate to the mesenteric veins around the bladder (*S. haematobium*) or intestine (*S. mansoni* and *S. japonicum*) and produce eggs. Eggs that become entrapped in tissues of the definitive host induce an immune-mediated response, leading to inflammation, granulomatous changes and subsequent fibrosis (Rollinson, 2009). Clinical manifestations of chronic schistosomiasis include abdominal pain, enlarged liver, blood in stool or urine (depending on species) (Caffrey, 2015; Colley et al., 2014) and the development of squamous cell carcinoma in humans with chronic *S. haematobium* infection (Palumbo, 2007; Rollinson, 2009).

### 5.2. Parasitic roundworms (nematodes)

Worms of the phylum Nematoda can cause important parasitic diseases of animals. For instance, species of *Haemonchus*, *Teladorsagia*, *Ostertagia* and *Trichostrongylus* (order Strongylida) are common pathogens of ruminants (mainly affecting cattle, sheep and goats) and represent a substantial economic burden to livestock industries (Charlier et al., 2014; Loyacano et al., 2002; Sutherland and Scott, 2009), with economic losses estimated at tens of billions per year worldwide (Roeber et al., 2013). In Australia alone, losses caused by endoparasites in sheep are estimated at 436 million dollars annually (Lane et al., 2015).

Other parasitic nematodes have a detrimental effect on human health. For example, members of the genus *Trichinella* are food-borne

pathogens that infect humans and a wide range of domesticated and wild vertebrate animals, including mammals, reptiles and/or birds (Murrell and Pozio, 2000). Humans become infected when eating undercooked, inadequately cured or raw meat from an infected animal. Although trichinellosis represents a zoonotic disease of importance worldwide, given the broad host range of *Trichinella* spp. (see Devleesschauwer et al., 2015; Murrell and Pozio, 2011), a major burden to human health is caused by soil-transmitted helminths (STHs; Bethony et al., 2006; World Health Organization, 2012a).

STHs include the human whipworm *Trichuris trichiura*, which lives in the caecum and colon of humans. Infective eggs contaminating the soil are the source of infection when they are ingested, and then develop into adult worms inside the host gut. Female worms can produce up to 3000 eggs per day, which are shed *via* the faeces and disseminated in the environment (Bethony et al., 2006). These nematodes and other STHs usually have direct life cycles, i.e. they do not require an intermediate host to develop and/or reproduce. However, a period of development in the soil is required for eggs to become infective. Eggs are passed in the faeces and contaminate surrounding soil, enabling transmission.

NTDs caused by intestinal nematodes affect more than two billion people worldwide, predominantly in communities with poor infrastructure and without access to sufficient sanitation (Hotez et al., 2009; World Health Organization, 2012b). In the most impoverished areas of the world, many children are infected with multiple species of STHs, causing reduced growth and slowed intellectual development (Hotez et al., 2009; World Health Organization, 2012b), exacerbating poverty due to long-term ill health and decreased productivity.

## 6. Control of helminth infections and associated challenges

The substantial impact of parasitic helminths on the health of humans and animals means that the control of these pathogens is of utmost importance. Although a range of intervention measures, such as improved sanitation and removal of parasites from the environment, are employed, currently, in the absence of commercial vaccines against most helminths, the mainstay for the control of infections is the use of a small number of anthelmintics (Keiser and Utzinger, 2010; Prichard et al., 2012). Most of these drugs target components of the worms' nervous systems; for example, piperazine and macrocyclic lactones, such as ivermectin and moxidectin, target gamma-aminobutyric acid (GABA) receptors. Other compounds, including amino-acetonitrile derivatives (monepantel; Kaminsky et al., 2008a, 2008b), imidazothiazoles (levamisole), spiroindoles (derquantel) and tetrahydropyrimidines (pyrantel/morantel), target a different class of neurotransmitter receptors, the acetylcholine receptors (Holden-Dye and Walker, 2014). Additionally, cyclooctadepsipeptides (emodepside; Kulke et al., 2014) interact with calcium-activated potassium channels. While these drugs are mainly used for the treatment of nematode infections, the control of flatworms often relies on two key drugs, praziquantel (Caffrey, 2015) and triclabendazole (Caffrey et al., 2012). The latter belongs to the class of benzimidazoles, which also includes albendazole and mebendazole (Keiser and Utzinger, 2010). Anthelmintics of this class target the cytoskeleton of the parasite by binding to β-tubulin and thus inhibit the formation of microtubules (Holden-Dye and Walker, 2014; Lacey, 1990).

Although drugs for the treatment of most helminth infections are available and their widespread use in mass drug administration (MDA) programmes for humans has led to a substantial reduction of some NTDs in several geographical areas (Prichard et al., 2012; Rollinson et al., 2013), challenges are associated with the use of these drugs.

First, worms of humans often affect the poorest of communities and, therefore, drugs need to be supplied to people at no cost (Hotez et al., 2009). Furthermore, MDA programmes need to provide education and training for medical staff in affected areas, and ensure efficient medicine logistics, administration and treatment monitoring (Webster et al., 2014; World Health Organization, 2012b); for example, some drugs have substantial side effects, resulting in poor patient compliance (Bethony et al., 2006). In the veterinary field, efforts aim to educate farmers about the correct timing, type and dosage of anthelmintic treatment to minimise the inefficient use of these drugs (Besier and Love, 2012; Besier, 2012). In addition, consumers' preference for minimal chemical intervention in animal production has recently restricted the use of anthelmintics (Knox et al., 2012).

Although these challenges are affecting the efficacy of intervention programmes, the main compounding factor of effective disease control is resistance against currently available anthelmintics. In livestock animals, many economically important parasites have developed resistance against most anthelmintics within a relatively short period of time after their introduction (Jabbar et al., 2006; Kaplan, 2004; Rose et al., 2015; Scott et al., 2013; Wolstenholme et al., 2004; Wolstenholme and Kaplan, 2012).

Human MDA programmes face similar issues, with a risk of resistance developing (Vercruysse et al., 2011; Webster et al., 2014), and the reliance on single drugs (e.g., the use of praziquantel for treating schistosomiasis) inducing a selective pressure that could favour resistant parasite populations. Low efficacy and cure rates have been reported both in laboratory and field settings for a range of different human helminths, including schistosomes, hookworms (*Necator americanus* and *Ancylostoma* spp.), common roundworms (*Ascaris lumbricoides*) and whipworms (*Trichuris trichiura*) (Keiser and Utzinger, 2008; Olsen et al., 2009; Soukhathammavong et al., 2012; Wang et al., 2012), but the presence or emergence of treatment-refractory parasite populations has not yet been reported (Keiser and Utzinger, 2010). Nevertheless, it is possible that with the continued 'pressure' from MDA, the resistance situation known in the veterinary field might also unfold in parasitic worms of humans (Bergquist et al., 2017; Webster et al., 2014).

## 7. Anthelmintic drug discovery using genomics and transcriptomics

Given challenges with the chemical control of parasitic worms of humans and other animals, there is a need for sustained efforts to identify and characterise new drug targets and to develop novel anthelmintics. In this context, several studies have focused on protein kinases, given their roles in many essential cellular signalling processes and/or in disease (Blume-Jensen and Hunter, 2001; Cohen, 2000, 2001), and the fact that they are amenable to targeting by small molecule drugs (Cohen, 2002; Cohen and Alessi, 2013). For example, Caffrey et al. (2009) inferred three protein kinases as potential drug targets in the blood fluke *S. mansoni* using a chemo-genomic filtering approach. Another study (Taylor et al., 2013), investigating drug targets in parasitic nematodes, inferred 68 protein kinases to be essential based on a comparison of kinomes among parasitic and free-living nematodes. Based on this essentiality prediction, the authors (Taylor et al., 2013) inferred these proteins to represent 'good' drug targets. Subsequently, the anthelmintic activity of 18 kinase inhibitors, targeting human homologs of the identified parasite kinases, were tested against *C. elegans*, *H. contortus* and *B. malayi* (see Taylor et al., 2013). Taken together, these studies provided promising starting points for drug discovery efforts.

In the search for new anthelmintics, the computational identification of kinase inhibitors has gained momentum (Panic et al., 2014; Preston et al., 2015). In this context, inhibitors that have been approved for the treatment of human diseases and/or that have shown potential to be repurposed as anthelmintics (Ekins et al., 2011) have been of particular interest. For example, in schistosomes, this so-called 'piggyback' approach has been employed extensively, and has resulted in the functional investigation of human kinase inhibitors as anti-schistosome agents (Beckmann and Grevelding, 2010; Beckmann et al., 2012; Buro et al., 2014; Dissous and Grevelding, 2011; Gelmedin et al., 2015; Morel

et al., 2014; Walker et al., 2014).

As these examples show, the repurposing of well-studied compounds is a viable option to find new starting points for drug development against parasitic helminths. However, it also illustrates that most studies focus on single genes/proteins that represent well-established drug targets in other organisms and have approved drugs associated with them. Clearly, to move from this 'gene-centric' to a more 'genome-centric' approach, there is a need for integrative tools and resources that enable the identification, classification and curation of protein kinases, to allow researchers to harness the large genomic, transcriptomic and functional data sets that are now publicly available.

## 8. Resources and databases for protein kinases

Attempts to integrate information on kinases from a range of resources to build a "Protein Kinase Resource" date back to before the human genome sequence was published (Niedner et al., 2006; Petretti and Prigent, 2005; Smith, 1999; Smith et al., 1997). A more recent, up-to-date, kinome database (KinBase; http://kinase.com/web/current/kinbase/; maintained by the Manning group at Genentech Inc., in collaboration with the Razavi-Newman Center for Bioinformatics at the Salk Institute for Biological Studies, and Cell Signaling Technology Inc.) contains the kinome data sets of 15 different species (Table 1), most of which represent model organisms or species at phylogenetic branch points. This resource is mainly a collation of individually published kinome projects, allows the retrieval of full-length amino acid sequences, catalytic domain sequences and kinase group, family and subfamily classifications, and provides a perspective of evolutionary relationships among kinase sequences. However, 209 of the 7597 sequences (2.75%) in KinBase are less than 200 amino acids long, suggesting that they are fragments of protein kinases. These numbers show that even the best-curated kinase database contains draft or un-curated data, a statement that is further supported by differing numbers of kinases reported in different studies for the same organisms (Supplementary Table 1).

The "Protein Kinase Ontology Browser" (ProKinO; Gosal et al., 2011; McSkimming et al., 2015) represents an ontological framework that integrates a wide range of information on the sequences, structures, functions, mutations and pathways of protein kinases. By using a defined vocabulary and ontology, this system allows integrative analyses of protein kinases to be performed, which can aid in the formulation of testable hypotheses. The authors (Gosal et al., 2011; McSkimming et al., 2015) demonstrated the utility of ProKinO by mining and annotating the human cancer kinome. Although this platform contains kinase information on 15 diverse species (including *C. elegans*), it contains relatively sparse information on individual protein kinases for all species, except for *H. sapiens*; this resource mainly serves the human cancer research community. A recent expansion of ProKinO termed KinView (McSkimming et al., 2016) further supports this notion by providing an interactive 'visualisation' interface facilitating comparative analyses between subsets of kinases (e.g., families/subfamilies) and integrating other information, including natural sequence variation, cancer variants, post-translational modifications and residue-specific annotation. Another example of such a specialised resource is KinMutBase (Ortutay et al., 2005), which contains information on disease-causing variations in human protein kinase domains.

In addition to databases containing information on protein kinases, PhosphoSitePlus (Hornbeck et al., 2015) and PhosphoPOINT (Yang et al., 2008) contain data on post-translational protein modifications (PTM), including phosphorylation sites, and protein-protein interactions (PPI), presenting important 'interactome' resources for protein kinases. Another database, "Kinase Pathway Database" (Koike et al., 2003), employs natural language processing to extract and integrate information from published articles to create a knowledgebase on signalling pathways of protein kinases. Although these resources are useful in the broader context of kinase research, they are mostly restricted to the analysis of human kinases, and do not provide kinase identification or classification capabilities.

## 9. Methods commonly used for the characterisation and annotation of kinomes from draft genomes

Given the expanding number of available complete and draft genomes, there is a clear need for automated tools for high-confidence identification and classification of protein kinases. To address this need, several software packages have been developed for this purpose. Historically, the identification of protein kinases in sequence repositories relied on sequence similarity searches using the "Basic Local Alignment Search Tool" (BLAST; Altschul et al., 1997). However, kinases may exhibit substantial sequence divergence in regions outside of the conserved kinase catalytic domain, resulting in low-similarity matches (BLAST scores).

To address this limitation, hidden Markov models (HMMs) have been employed for protein domain detection (Eddy, 1996). HMMs are frequently used for pattern detection in the context of speech recognition (Krogh et al., 1994), but it has become clear that these models are readily applicable to the detection of functional protein domains or conserved nucleotide patterns. They are statistical descriptors of sequence conservation inferred from multiple sequence alignments (MSAs) and have been shown to outperform standard local alignment-based methods for sequence searching/comparisons (such as BLAST), both in terms of sensitivity and specificity (Sonnhammer et al., 1997). A collection of HMMs for most protein domains, including two models representing sequences of the catalytic domain of serine/threonine kinases (Pkinase; PF00069) and tyrosine kinases (Pkinase_Tyr; PF07714), are stored in the Pfam database (Sonnhammer et al., 1997). The application of these two Pfam HMMs allows for the subdivision of protein kinases in two major classes: kinases that phosphorylate serine and/or threonine residues and kinases that phosphorylate tyrosine residues.

Other methods have been developed and have improved the classification of protein kinases. For instance, Kinomer (Martin et al., 2009; Miranda-Saavedra and Barton, 2007) is a computer program that improves the classification of kinases by constructing and applying 12 group- or family-specific HMMs. This approach facilitated the classification of some previously unclassified kinases. The Kinomer HMM library outperforms protein BLAST-based approaches (Camacho et al., 2009) and the general Pfam HMMs in both the identification and group-level classification of protein kinases (Martin et al., 2009). This improved accuracy is facilitated by group-specific HMMs compared with a single HMM for the entire protein kinase superfamily, recognising homologs with higher scores and rejecting non-homologs with higher E-values (Miranda-Saavedra and Barton, 2007). This method has been applied to the characterisation of more than 40 draft kinomes, including those of amoebae (Clarke et al., 2013), apicomplexans (Talevich et al., 2014), microsporidia (Miranda-Saavedra et al., 2007) and other fungi, algae, plants and vertebrates (Martin et al., 2009). The major limitation of Kinomer is that it can only classify kinases to the group level and has limited capabilities for the classification of aPKs, only being able to identify protein kinases within four of at least 13 families. Further classification of kinases into subfamilies cannot be achieved using this tool, and novel families/subfamilies are likely to be missed or not classified beyond the group level.

In contrast, the program Kinannote (Goldberg et al., 2013) produces a draft kinome and comparative analyses for a predicted proteome using a single-line command, and is currently the only tool that automatically classifies protein kinases to the sub-family level using the controlled vocabulary of Hanks and Hunter (Hanks and Hunter, 1995; Hanks et al., 1988). In a first step, Kinannote employs an ePK HMM derived from a manual alignment of the *Dictyostelium* kinome (Goldberg et al., 2006). By using a model that represents an early evolutionary branch point and employing a relaxed cut-off, this first filtering step aims to reduce the search space for following steps, while retaining

sensitivity to evolutionary divergent kinase sequences. Subsequently, a position-specific scoring matrix (PSSM; cf. Henikoff and Henikoff, 1996), built from an MSA of protein kinase domains of the *Dictyostelium* kinome and other kinomes in KinBase, is employed to identify conserved sequence motifs in the kinase catalytic domain that are important for kinase activity. The resultant score is then applied in the second phase of the algorithm, together with results of a search against KinBase using BLAST. In this phase, kinases are identified based on sequence similarity to kinases in KinBase, as well as HMM and PSSM scores. Depending on these scores, the sequences are either retained for subsequent classification *via* a search against KinBase using BLAST, or are added to the draft kinome set for further curation, and designated as either 'twilight' hits or "protein kinase subdomain-containing proteins". Sequences without BLAST hits and scores below the defined cut-off values are considered not to represent kinases and are thus excluded from further analysis.

Based on published evidence, Kinannote out-performs Kinomer, in terms of sensitivity (Goldberg et al., 2013), is user-friendly and can rapidly process a large number of amino acid sequences. Furthermore, the Kinannote output is human-readable, providing a foundation for manual curation of sequence data. Nevertheless, this tool has several limitations. First, it only accepts protein sequence data as input and does not allow for additional information, such as RNA-sequence (RNA-Seq) evidence or genomic data, to be provided that could aid subsequent curation. Second, recently diverged kinase sequences might not be identified using the *Dictyostelium* HMM, even when a relaxed cut-off is employed. In addition, most aPKs are not detected by the HMM because it is built for ePKs, and will thus only detect a small number of aPKs with sequences similar enough to those of ePKs. Third, the BLAST-based classification approach is unable to (fully) classify divergent/novel families/subfamilies (e.g., those of parasitic helminths) as they are not represented in KinBase.

Some of these limitations can be overcome by employing orthology-(Li et al., 2003) or phylogeny-based (Yang and Rannala, 2012) approaches. For example, a phylogenetic analysis of the kinome of the malaria parasite, *Plasmodium falciparum* (see Ward et al., 2004) revealed a novel kinase family with a conserved Phe-Ile-Lys-Lys (FIKK) motif, which, upon investigation of additional apicomplexan kinomes (Talevich et al., 2011), was shown to be common among, yet specific to, many apicomplexans. Additionally, kinases can be grouped based on the presence of particular accessory domains and their order within the amino acid sequence (Hanks, 2003; Manning et al., 2002a, 2002b), for example, by employing domain search tools, such as InterProScan (Jones et al., 2014). However, to our knowledge, this approach has, until recently, not been applied to support the global classification of protein kinases.

Other approaches, such as that employed in the "Conserved Domain Database" (CDD; Marchler-Bauer et al., 2013), rely on experimentally determined three-dimensional structures of kinases to define functional domains. Although this strategy can allow for the identification of divergent kinase sequences with conserved structures, it does not achieve family or subfamily classification. In contrast, computational structural homology modelling of unclassified kinases (e.g., using the program I-TASSER; Yang et al., 2015) could provide clues for the classification of kinases into families/subfamilies. In addition, kinase sequences can be further characterised based on the presence and characteristics of phosphorylation sites (Hornbeck et al., 2015), or based on their predicted catalytic activity (Boudeau et al., 2006; Murphy et al., 2014).

Taken together, although many tools for the identification, classification and annotation of protein kinases exist, until recently, there has been no tool that combines and integrates all of the described methodologies to achieve a reliable, comprehensive classification, which can be universally applied to kinomes from a broad range of genetically diverse organisms. This situation represents a challenge for the annotation of helminth kinomes, particularly because most HMM-based methods and databases do not include helminth sequences in their model-building steps. Furthermore, none of the tools described here provides outputs that readily enable detailed, automated or semi-automated curation of amino acid and nucleotide sequences inferred from draft genomes.

## 10. Helminth kinomes predicted from draft genomes, and limitations of the methods used

Most published draft genome projects of parasitic worms provide an estimate of the number of encoded protein kinases (Table 2), and sometimes include classification of kinases into the recognised groups families and/or subfamilies. However, these estimates vary for the same species among different publications, and depend on the methodology used for identification and classification of protein kinases. In some cases (e.g., Bennuru et al., 2016; Desjardins et al., 2013), the authors note that additional curation of kinome data sets is required in the future, due to potentially inaccurate or incorrect gene models and/or misclassifications. Most studies do not extend their kinome analyses beyond the identification of the number of protein sequences containing a kinase catalytic domain. Such analyses are usually conducted either based on a match to one of the two HMMs representing this domain in the Pfam database (PF00069 and PF07714) or based on matches to known protein kinase sequences in a database (e.g., UniProtKB/Swiss-Prot; Boutet et al., 2007) using BLAST. Although a relatively large number of such estimates are available for draft genomes of parasitic worms, until recently, no well-curated kinomes have been published in their own right, with the exception of the eukaryotic protein kinase complement of *S. mansoni* (see Andrade et al., 2011). However, the latter study did not identify or classify divergent PKLs/aPKs, such as RIO kinases. Investigating these divergent sequences in parasites is warranted, given that they might represent parasite-specific drug targets or could provide important clues regarding the unique biology of a parasite (Breugelmans et al., 2014; Campbell et al., 2011).

In addition to the *S. mansoni* kinome, draft kinomes for two parasitic nematodes (*Loa loa* and *Wuchereria bancrofti*) have been defined (Goldberg et al., 2013) employing the automated classification method in Kinannote and subsequent refinement based on sequence similarity with the kinases of *D. discoideum*, *S. cerevisiae*, *D. melanogaster* and *H. sapiens*, and orthology with *C. elegans* kinase sequences. Furthermore, as part of the *L. loa* genome project (Desjardins et al., 2013), the draft kinomes of *L. loa*, *B. malayi*, *W. bancrofti*, *Ascaris suum*, *Pristionchus pacificus*, *Meloidogyne hapla* and *Trichinella spiralis* were identified based on orthology with *C. elegans* sequences and/or a protein kinase HMM representing *Dictyostelium* ePKs (Goldberg et al., 2006). These two studies reported substantially different kinomes for both *L. loa* and *W. bancrofti*, indicating the significant influence of the two different methods employed on the resultant kinome data sets. Other studies have not defined whole draft kinomes, but used Kinomer and a BLAST-based approach to define a subset of kinases as potential drug targets (Taylor et al., 2013) and to study a single signalling pathway in *S. japonicum* (see Wang et al., 2006), respectively.

Although all of these studies have provided useful starting points for functional investigations, detailed work on worm kinomes and associated aspects has been compromised by a lack of or inadequately curated data.

## 11. Recent progress in the curation of worm kinomes using advanced bioinformatic workflows

Based on recent work and experiences made in kinome curation projects for seven helminth species (Stroehlein et al., 2015a, 2015b, 2016, 2017), we have now established an advanced bioinformatic pipeline for the comprehensive identification, curation, classification and functional annotation of the full complements of protein kinases in draft genomes of parasitic worms. This pipeline has proven to be practical, accurate and time-efficient, addresses some of the limitations of

**Table 3**
Curated kinomes of parasitic helminths and number of predicted kinase drug targets.

| Species | Eukaryotic protein kinases (ePKs) | Atypical and protein kinase-like protein kinases (aPKs/PKLs) | Number of predicted/prioritised drug targets | Reference(s) |
|---|---|---|---|---|
| *Schistosoma haematobium* | 261 | 8 | 40 | (Stroehlein et al., 2015a) |
| *Schistosoma mansoni* | 259 | 8 | n.r. | (Andrade et al., 2011; Stroehlein et al., 2015a) |
| *Haemonchus contortus* | 386 | 46 | 13 | (Stroehlein et al., 2015b) |
| *Trichinella spiralis* | 205 | 21 | n.r. | (Stroehlein et al., 2016) |
| *Trichinella pseudospiralis* | 212 | 20 | n.r. | (Stroehlein et al., 2016) |
| *Trichuris suis* | 216 | 65 | n.r. | (Stroehlein et al., 2017) |
| *Trichuris trichiura* | 218 | 71 | n.r. | (Stroehlein et al., 2017) |

n.r., not reported

available tools, and overcomes challenges inherent to the analysis of different genomic and transcriptomic data sets (see Section 10). Employing this pipeline, we have curated 1996 protein kinase sequences representing seven diverse worm species (Table 3). This provides the largest resource of well-curated kinases and serves as a foundation to explore kinase signalling in worms, compare kinomes among species and investigate protein kinases as targets for anthelmintic drugs.

### *11.1. Characterisation of kinomes of helminths that are distantly related to well-curated model organisms*

Pipelines and tools for kinase identification often rely on stochastic models (e.g., HMMs and PSSMs), which allow for sensitive and specific searches for conserved functional domains (Goldberg et al., 2013; Martin et al., 2009). However, sequences from species that are phylogenetically distinct from those representing the 'seed' alignment forming the HMMs for kinase catalytic domains (Pkinase, PF00069 and Pkinase_Tyr, PF07714) might not be identified due to a lack of sensitivity of such HMMs. Therefore, when defining the *S. haematobium* kinome (Stroehlein et al., 2015a), we used the Pfam HMMs representing kinase catalytic domains to interrogate a range of trematode genomes and then constructed new, trematode-specific HMMs based on the identified sequences for each kinase group, which revealed a higher sensitivity compared with the canonical Pfam HMMs.

However, for atypical kinases, we could only construct robust trematode-specific HMMs for two families by employing Kinannote for initial identification of these kinases and subsequently inferring HMMs from MSAs. Kinannote can detect and annotate some atypical protein kinases but misses divergent sequences (Goldberg et al., 2013). For the two schistosomes studied (*S. haematobium* and *S. mansoni*), this tool was able to classify kinases within the families ABC and RIO, but there was a lack of evidence for sequences in other atypical protein kinase families, including BRD, PDHK, PIKK and TAF1. In contrast, for kinomes of five other parasitic worms investigated recently (Stroehlein et al., 2015b, 2016, 2017), Kinannote identified and classified some kinases in these families. Therefore, it is unlikely that the apparent lack of these kinase sequences in schistosomes is related to the use of the less sensitive method implemented in Kinannote, but rather represents a loss of these kinases or a substantial heterogeneity in their amino acid sequences that precluded their identification.

In a recent study (Stroehlein et al., 2017), an improved and more sensitive identification and classification approach, based on conserved sequence domains, led to an increase in the number of predicted and classified atypical kinase and kinase-like sequences, which could now be applied to the kinomes of schistosomes to improve our understanding of this intriguing group of enzymes and to lend support to the proposal that schistosomes and/or other species of flatworms lack some atypical kinases (Stroehlein et al., 2015a). Taken together, our approach based on trematode-specific HMMs, followed by a strategy that integrates comparative pairwise genomics and phylogenetic analyses, allowed us to define and curate the *S. haematobium* kinome, which comprises 269 kinases representing all nine major kinase groups. A subsequent comparison of the kinome of *S. haematobium* with that of *S. mansoni* revealed, despite high overall sequence conservation, some differences in the number of encoded kinases, which could be linked to the pathogens' unique biology and predilection sites in the human host (cf. Stroehlein et al., 2015a).

Subsequent analysis of the developmental transcription profiles of kinase genes suggested that the majority of schistosome kinases play essential roles in signalling processes in all developmental stages studied, whereas some kinase genes were sex- or stage-specifically transcribed and could be assigned to specialised functional categories such as spermatogenesis, stress response and muscle development. From an applied perspective, data from both the transcriptional analysis and the pairwise kinome comparison was integrated into an advanced drug target prediction pipeline. This pipeline was used to prioritise 40 *S. haematobium* kinases as potential drug targets. Subsequently, 42 drug-like compounds were predicted to bind one or more of these kinases based on sequence homology to validated targets. Predicted compounds can now be prioritised further (e.g., based on their chemical properties or using *in silico* docking experiments) and then assessed as to their ability to disrupt schistosome development and/or viability *in vitro*, offering a prospect for the design, development and/or repurposing of kinase inhibitors to schistosomes.

### *11.2. Improved pairwise curation and drug target prediction employing a well-curated kinome and extensive transcriptomic data*

In contrast to the approach employed for the identification and classification of the kinomes of schistosomes, the relatively close taxonomic relationship between the parasitic nematode *Haemonchus contortus* and the free-living model organism *C. elegans* (Rhabditina; clade V; see Blaxter and Koutsovoulos, 2015) allowed for a more straight-forward identification and classification of kinases in the former species, using conventional Pfam HMMs and the well-curated kinome of *C. elegans* (see Stroehlein et al., 2015b). Taken together, we identified, curated and classified 432 kinases representing all recognised kinase groups and, by extending the previously established approach (Stroehlein et al., 2015a) and integrating high-quality transcriptomic data, we considerably improved the prediction and annotation of kinase genes encoded in the *H. contortus* genome. A comparison of the kinomes of *H. contortus* and *C. elegans* revealed considerable variation in the numbers of particular kinase families and subfamilies, which likely relate to differences in biology, life cycle and habitat between these worms. In contrast, the relatively conserved parts of the kinase complements of *H. contortus* and *C. elegans*, provide unique opportunities to explore the functions of these enzymes in *H. contortus* and to gain an improved understanding of the underlying molecular processes or mechanisms that regulate development, reproduction and physiology in this parasite.

The relatively high degree of amino acid sequence conservation between these two species also facilitated a more comprehensive and

reliable inference of functions and pathway associations for *H. contortus* compared with species whose amino acid sequences are less similar to orthologs in the arguably best-studied model organism, *C. elegans* (see Harris et al., 2014). This information can now be employed to assist in the reconstruction of biochemical pathways in *H. contortus* (cf. Gilabert et al., 2016; Mohandas et al., 2016). However, given that *C. elegans* is entirely free-living and *H. contortus* has both free-living and parasitic stages, we deemed it likely that there are differences in biochemical kinase signalling pathways. Indeed, we detected seven *H. contortus* protein kinase sequences without a *C. elegans* homolog, and reported several other quantitative and qualitative adaptations in the kinome of *H. contortus* compared with *C. elegans* (see Stroehlein et al., 2015b). In this context, we employed an improved strategy (as implemented in the "domain architecture" search tool IDA; https://www.ebi.ac.uk/interpro/search/domain-organisation) for kinase characterisation and functional annotation; unclassified kinase sequences were functionally annotated based on the presence of all domains and/or signatures determined by InterProScan (Jones et al., 2014), and their order within the kinase sequence ("domain architecture"). The improved functional annotation of kinases, together with transcription analysis across key developmental stages (egg, L1, L2, L3, L4 and adult) and both sexes (for L4 and adult) of *H. contortus*, enabled us to infer central roles in developmental and reproductive processes for suites of kinase genes selectively transcribed in particular developmental stages of *H. contortus*.

Next, we modified the filtering approach established for the prediction of drug targets in *S. haematobium* by adding additional criteria and employing a ranking strategy. Taken together, we prioritised a set of 13 kinase drug targets and a total of 1517 small-molecule compounds predicted to bind to them. These results provide starting points for targeted screening and anthelmintic discovery efforts against *H. contortus*. Furthermore, the curated kinome data should provide a useful resource for fundamental investigations of kinases and signalling pathways in this nematode.

### *11.3. Exploration of enoplean kinome biology employing a consolidated kinase curation and annotation pipeline*

Recently, we defined and curated the kinomes of four other species of parasitic nematodes (*Trichinella spiralis*, *Trichinella pseudospiralis*, *Trichuris suis* and *Trichuris trichiura*; see Stroehlein et al., 2016, 2017). These species belong to the class Enoplea and assume a unique taxonomic position within the phylum Nematoda compared with other parasitic and free-living nematodes (class Chromadorea; cf. Blaxter and Koutsovoulos, 2015; Blaxter et al., 1998; Korhonen et al., 2016; Roberts and Janovy Jr., 2009). Our analyses revealed that the kinomes are all remarkably 'compact' (Table 3), representing the smallest curated metazoan kinomes to date. This substantial difference in kinome size compared with other curated nematode kinomes might be explained by the unique biology of these enoplean species with respect to chromadoreans. Enopleans might control intra- and inter-cellular signalling in an efficient way that is distinct from other metazoans, and thus only involves a fraction of kinases present in signalling pathways in other organisms. It is also conceivable that some species, and *Trichinella* in particular (cf. Stroehlein et al., 2016), rely on host cell pathways for some signalling processes and have lost the orthologous kinase genes from their genomes.

The strategy employed for the characterisation of these four enoplean kinomes relies on the previously established reciprocal, pairwise curation approach (Stroehlein et al., 2015b), but has been extended and enhanced by integrating three-dimensional modelling data, facilitating the reliable prediction of a previously uncharacterised N-terminal domain in an apparently enoplean-specific kinase for all four species. Additionally, for the characterisation of the *T. suis* kinome, the availability of extensive transcriptomic data enabled the application of an improved strategy for the re-prediction of gene models (Stroehlein et al., 2017). The mapping of the *de novo*-assembled transcripts from multiple developmental stages to the genomic scaffolds created a robust data set that enabled the subsequent re-assembly (using CAP3; Huang and Madan, 1999) and inference of open reading frames. This addition substantially improved the pipeline. Since this step was computationally tractable, it was fully automated, which substantially sped up the confirmation of gene predictions and reduced erroneous gene models. However, the success of this approach hinges on the number of RNA-Seq libraries (and accordingly, the quantity of RNA-Seq reads) available for an organism. For example, the re-prediction of gene models of the related *Trichuris trichiura* kinome was mainly achieved by transferring the high-confidence gene models from *T. suis* to *T. trichiura* because *de novo*-assembled transcripts of *T. trichiura* could not be re-assembled and improved, did not cover predicted kinase genes or were of insufficient quality (Stroehlein et al., 2017).

The transcriptomic data available for *T. suis* allowed us to investigate the transcription of kinase genes across developmental stages, sexes and tissues, which revealed that selectively transcribed genes can be linked to central roles in developmental and reproductive processes, some being associated with pathways involved in the communication of the parasite with its environment.

We also employed an enrichment analysis to investigate links between pathway association, classification or functional domains of kinase sequences and the transcription profile of their genes. Although this analysis revealed some enriched kinase families in adult male *T. suis*, which could be linked to male-specific developmental/reproductive processes, it also highlighted the limitations of such an approach for most parasitic species. Most enrichment analyses were impaired by a relatively low percentage of kinases that could be assigned to a pathway and/or specific GO terms, even for species that are more closely related to the model organism *C. elegans* (cf. Harris et al., 2014), such as *H. contortus*. Nevertheless, most of the kinases that could be assigned to a pathway had at least one ortholog in *C. elegans*, suggesting that kinase sequences that are conserved between *C. elegans* and parasitic nematodes are more likely to be accurately assigned to a pathway. This finding emphasises the need for a more detailed analysis and curation of pathways in parasitic nematodes (cf. Gilabert et al., 2016; Mohandas et al., 2016) and the integration of such data into pathway databases, such as the Kyoto Encyclopedia of Genes and Genomes (KEGG; Kanehisa and Goto, 2000).

However, for most parasites investigated, such an analysis was partially hindered by data not being available for some developmental stages (e.g., due to challenges obtaining such material), or developmental stages being represented by single samples. Without sufficient biological replicates, statistical enrichment analysis of transcripts for a particular developmental stage is hardly feasible, hampering the inference of biological meaningful information. Nevertheless, findings for *S. haematobium*, *H. contortus* and *T. suis* showed that kinase genes undergo substantial transcriptional regulation throughout parasite life cycles, and some of the observed variation was suggestive of roles in reproduction, development and morphological changes (Stroehlein et al., 2015a, 2015b, 2017). In the future, it would be beneficial to obtain RNA-Seq data for other stages and/or tissues (including replicates) for species (e.g., *Trichinella* spp.) for which only a limited number of stages have been studied.

Taken together, the characterisation and curation of kinomes for five 'divergent' nematode species (representing the Enoplea and Chromadorea) allowed for a global qualitative and quantitative comparison of their curated kinomes (Stroehlein et al., 2017). This analysis identified orthologous groups that were unique to the two *Trichuris* spp. investigated, those that were absent from all three enoplean species and were present only in the two rhabditids studied, and others that were specific to all enoplean species. Despite this diversity and differences in sequence similarity and domain architecture among the kinomes, we found 126 orthologous groups that contained representative kinase sequences of all five nematode species investigated. These findings and the differences between the kinomes of chromadorean and enoplean

species reflect evolutionary alterations that might be linked to adaptations in host-parasite interactions and environmental signalling processes.

Such analyses can elucidate the relationship between novel, parasite-specific families that cannot be classified into any of the existing families and/or subfamilies, and kinases with known classifications. This approach provided important information about the evolution of protein kinase families/subfamilies and about the potential functions of novel kinases. One such example is the nematode-specific KIN16 family (Manning, 2005; Morgan and Greenwald, 1993); sequences of parasitic nematodes analysed consistently formed orthologous clusters with epidermal growth factor receptor sequences encoded in the human and *C. elegans* genomes (Stroehlein et al., 2015b, 2016, 2017), indicating a closer relationship among these sequences than previously assumed (Manning, 2005). Another example of the utility of this approach was the identification of a MOS-like kinase sequence in the four enoplean kinomes analysed (Stroehlein et al., 2016, 2017). Phylogenetically, the human MOS kinase catalytic domain clusters with that of the MLKL protein sequence (Manning et al., 2002b). However, for parasitic nematodes the MOS-like sequence did not form an orthologous cluster with the human MLKL or MOS sequence, which led to a further investigation of this unusual protein sequence and a prediction of its three-dimensional structure (Stroehlein et al., 2016, 2017).

For other sequences that have diverged from their orthologs in other, non-nematode genomes, the combination of a controlled vocabulary and an orthology- and phylogeny-based approach allowed for them to be automatically assigned to the recognised groups, families and subfamilies. A similar approach has been previously applied (Desjardins et al., 2013) to achieve an improved kinase classification for a range of nematode kinomes. The program Kinannote was published shortly after by the same authors who reported two kinomes of filarial nematodes (*Loa loa* and *Wuchereria bancrofti*; see Goldberg et al., 2013) that had been investigated earlier (Desjardins et al., 2013). The kinase complements of these two filarial nematodes differed considerably between the two studies, which likely relates to the distinct orthology-based curation approaches employed.

Taken together, our curation and annotation pipeline represents an improved and practical tool for comprehensive kinase classification, by integrating a pairwise reciprocal curation step, Kinannote and an orthology- and phylogeny-based approach. In addition, it addresses some of the shortcomings of previous methods (e.g., Kinomer; Martin et al., 2009 and Kinannote; Goldberg et al., 2013) and has enabled the most comprehensive characterisation of protein kinase sequences of parasitic worms to date.

Results from these studies (Stroehlein et al., 2015a, 2015b, 2016, 2017) add evidence to potentially novel and/or biologically interesting proteins, which should now allow researchers to formulate new and intriguing hypotheses regarding the function of protein kinases in parasitic worms. For example, our findings suggest that enopleans have adapted and developed distinct molecular means of environmental sensing, and support roles for kinases in the regulation of host-parasite interactions, such as establishment of infection, host immune modulation, chemotaxis and/or localisation of nutrient sources. These results and the first findings from comparative studies of five diverse nematode kinomes serve as a framework for further detailed explorations of kinase signalling and evolution in nematodes. This and future work should provide a useful resource for investigating this important class of enzymes in worms and might pave the way for the identification of selected kinases as drug targets in some of the most socioeconomically important parasites.

## 12. Concluding remarks

In the context of expanding curated kinome data sets for many helminth species to facilitate global kinome comparisons, some of the challenges inherent to the analysis of kinases in draft genomes might be overcome in the future through the application of novel technologies, such as third-generation, long-read sequencing (Reuter et al., 2015; Roberts et al., 2013). Such technologies could help resolve genomic regions that are notoriously difficult to assemble (Reuter et al., 2015; Roberts et al., 2013). More contiguous assemblies would lead to improved gene predictions, which would facilitate a more comprehensive annotation of inferred protein sequences, including kinase classification as well as domain and pathway annotation.

Additionally, hypotheses regarding the fundamental biology of parasitic worms that were formulated in recent studies can now be tested experimentally by applying advanced, “systems biology” strategies. Such approaches would allow for the integration of available curated data on protein kinases and would facilitate the global analysis of cellular signalling mechanisms beyond that of protein phosphorylation alone. Importantly, such experiments would greatly enrich the present data sets, because they would provide novel evidence for the predictions made, and would help to validate and/or further improve some of the computational approaches established and/or employed here. For instance, transcriptomic profiles of kinase genes in different developmental stages and/or tissues of parasitic worms (e.g., *S. haematobium*, *H. contortus* and *T. suis*) could be further corroborated by sequencing and characterising small, non-coding RNAs (cf. Bai et al., 2014; Claycomb et al., 2017; Ma et al., 2016) or by employing proteomic approaches (cf. Dewalick et al., 2011; Hong et al., 2013; Sotillo et al., 2015; Zhang et al., 2013). In addition, the excretory/secretory (ES) proteins predicted for some worms (e.g., *Trichinella* spp. and *Trichuris* spp.) could also be experimentally investigated by proteomic studies (cf. Chaiyadet et al., 2016; Cortes et al., 2016; Liu et al., 2009; Robinson and Connolly, 2005; Robinson et al., 2005), guided by the predictions made recently (Stroehlein et al., 2016, 2017). Another potential avenue would be the identification of phosphorylated proteins in these parasites (phosphoproteomics), as previously reported for *S. japonicum* (see Cheng et al., 2013) and *Pristionchus pacificus* (see Borchert et al., 2012). In the context of kinase characterisation, this would provide important clues regarding the substrates of kinases and, thus, would aid in the deconvolution of signalling pathways. To gain further insight into the interaction among signalling proteins in these pathways, proteomic approaches could also be employed to identify and characterise protein complexes (Rigaut et al., 1999). Information gleaned from the application of these technologies would enhance our understanding of signalling in parasitic worms and would inform future drug discovery efforts, which could be further aided through the use of a chemical proteomics assay for kinase inhibitor profiling (Medard et al., 2015).

In addition to such proteomic experiments, the application of chromatin immunoprecipitation followed by massive parallel sequencing (ChIP-Seq; cf. Cosseau et al., 2009; Cosseau and Grunau, 2011; Roquis et al., 2015) and/or the global analysis of DNA methylation by bisulfite sequencing (MethylC-Seq; cf. Gao et al., 2012; Urich et al., 2015), would facilitate studies of the epigenetic mechanisms regulating gene expression and enhance the understanding of gene regulation across different life cycle stages and tissues of helminths.

Another interesting area for future studies would be the quantitative analysis of the complement of small-molecule, non-proteinaceous metabolites in biological samples *via* mass spectrometry (MS) or nuclear magnetic resonance spectroscopy (NMR) (Dettmer et al., 2007; Markley et al., 2017; Roessner and Bowne, 2009). Although metabolomic investigations of unicellular eukaryotic parasites currently predominate the literature (reviewed in Preidis and Hotez, 2015; Vincent and Barrett, 2015), there is a growing number of metabolomic studies of helminths, some of which have provided novel insights into the interaction between parasites and their host environment *via* excreted metabolites (Laan et al., 2017); how metabolic profiles in host tissues are altered during infection (Nishina et al., 2004; Saric et al., 2010); and how worms sense environmental and/or organismal cues (Hsueh et al., 2017). Although the latter study was conducted in the free-living nematode *C. elegans*, a similar approach could be applied to parasitic

helminths, to study which environmental and/or host cues trigger host invasion, migration and/or parasite development and metamorphosis.

In addition to these potential avenues, other metabolomic approaches could also provide new insights into the biochemical composition of metabolites within cells or tissues (Prosser et al., 2014). The exploration of tissue or whole-parasite metabolomes could lead to new experimental evidence needed for the detection of novel and/or the reconstruction of canonical metabolic pathways. A combination of such a strategy with predicted pathway maps, based on sequence homology, as they have been constructed in the kinase studies reviewed here, would allow the systematic integration of biomolecular interactions at the protein and metabolite levels and the application of computational tools for network modelling, to gain new, fundamental insights into the biochemical signalling of parasites (Holmes, 2010; Roberts et al., 2009; Tabei et al., 2016; Yamanishi et al., 2015).

Additionally, from an applied perspective, such investigations would provide new evidence to inform drug discovery efforts, as they could facilitate the deciphering of the mode of action of a drug by monitoring the response of the parasite to chemotherapeutics (Creek and Barrett, 2014). In this context, metabolomics has also proven useful for characterising fractions and/or small molecules from natural products that have anti-parasitic activity (Holmes, 2010; Kumarasingha et al., 2016). Furthermore, metabolomic studies could inform essentiality predictions based on kinase pathway analyses conducted for *S. haematobium* and *H. contortus*. Importantly, this approach would greatly benefit from being combined with gene knockdown experiments (Dalzell et al., 2012; Maule et al., 2011), as it would allow new hypotheses regarding the function(s) of kinase genes in particular biochemical pathways to be tested.

While such knockdown experiments can be routinely carried out for unicellular eukaryotic parasites in a relatively high-throughput manner (cf. Alsford et al., 2011; Kolev et al., 2011; Morf et al., 2013), there has been variable success of RNAi-mediated knockdown for different species of parasitic worms (Geldhof et al., 2007; Knox et al., 2007; Maule et al., 2011; Viney and Thompson, 2008). Although extensive genomic and transcriptomic data sets have now enabled the identification of the complements of RNAi effector proteins in many parasitic nematodes (e.g., Dalzell et al., 2011; Schwarz et al., 2013), challenges in the application and experimental design of RNAi-mediated knockdown remain (Dalzell et al., 2012). However, several, more recent studies report the successful application of RNAi to a range of nematode species. For example, the expression of the immunomodulatory paramyosin-encoding gene was successfully silenced in *Trichinella spiralis via* soaking and electroporation techniques (Chen et al., 2012). For *H. contortus*, some promising studies also report gene silencing (Samarasinghe et al., 2011; Zawadzki et al., 2012), albeit success was dependent on the life cycle stage used, the RNAi delivery method and the tissue expression of the target genes. Other studies report the application of RNAi to the brown stomach worm, *Teladorsagia circumcincta* (see Tzelos et al., 2015), the large pig roundworm, *Ascaris suum* (see McCoy et al., 2015) and the entomopathogenic nematode *Heterorhabditis bacteriophora* (see Ratnappan et al., 2016). For schistosomes, there are also numerous reports of the successful application of RNAi (reviewed in Da'dara and Skelly, 2015; Hagen et al., 2012), and a recent study achieved persistent knockdown of selected genes using a lentivirus transduction approach (Hagen et al., 2014, 2015). The application of such a virus-based transduction system for parasitic nematodes would be a major advance and would, if successful, overcome the challenges associated with conventional RNAi approaches in these organisms (Dalzell et al., 2012; Maule et al., 2011). Extending results from recent computational predictions, this technique could be used to study functional roles of kinases and signalling pathways in development and reproduction of helminths, and would represent a powerful tool, generally, for functional genomic investigations. Furthermore, it would provide crucial experimental evidence to instil additional confidence into computational essentiality predictions and to explore some of the proposals made in recent studies regarding protein function. In addition to knockdown experiments to test the essentiality of kinase genes in parasites, the use of small-molecule chemicals to elicit lethal or sub-lethal phenotypes, would also carry merit, and could support essentiality and drug target predictions.

In conclusion, recent progress in the curation of kinomes of parasitic worms and prediction of drug target using advanced bioinformatics should guide functional and structural studies of kinases as well as the discovery of new anti-parasitic interventions - as biotechnology outcomes. Although the focus here has been on worm kinomes, the bioinformatic strategy and workflow established should be readily applicable to analyses of kinomes of any eukaryotic organism.

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## Appendix A. Supplementary data

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