Evolution of protein kinase signaling from yeast to man

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Protein phosphorylation controls many cellular processes, especially those involved in intercellular communication and coordination of complex functions. To explore the evolution of protein phosphorylation, we compared the protein kinase complements ('kinomes') of budding yeast, worm and fly, with known human kinases. We classify kinases into putative orthologous groups with conserved functions and discuss kinase families and pathways that are unique, expanded or lost in each lineage. Fly and human share several kinase families involved in immunity, neurobiology, cell cycle and morphogenesis that are absent from worm, suggesting that these functions might have evolved after the divergence of nematodes from the main metazoan lineage.

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Almost all eukaryotic protein phosphorylation is conducted by a single superfamily of eukaryotic protein kinases (ePKs) that share a conserved catalytic domain. Several atypical protein kinases (aPKs) are also known, which lack sequence similarity to ePKs, although some have structural similarity to ePKs [1–3]. ePKs are among the largest of protein families, comprising 1.5–2.5% of all eukaryotic genes. Within this superfamily, scores of distinct families have been conserved across species. The availability of the full yeast genome and the almost complete fly and worm genomes allows us to identify almost all protein kinases in these organisms and to explore the breadth of their protein phosphorylation pathways. With such kinome catalogs, we can assign orthology and use these model organisms to study human kinase function.

Using profile hidden Markov models, PSI-BLAST and homology-based gene prediction, we have created kinase catalogs for the yeast *Saccharomyces cerevisiae*, the nematode worm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* and we are also developing one for human ([4,5]; G. Manning and S. Sudarsanam, unpublished data at www.kinase.com/drosophila/; G. Manning *et al.*, unpublished data at www.kinase.com/human/kinome/; all further unpublished data can be seen at the latter website). To compare genes across such diverse species, we have developed a hierarchical classification of kinases into groups, families and subfamilies that is based on the taxonomy of Hanks and Hunter [6]. Where clear orthologs cannot be found, this classification allows the comparison of orthologous groups [7]. The classification is based primarily on kinase domain similarity, deduced from pairwise and multiple sequence alignments and phylogeny. Extra-catalytic sequence similarity and biological function were used to refine the classification. The full classification scheme, catalogs, sequences and sub-trees are available at http://www.kinase.com.

Family comparison (Fig. 1 and Table 1) shows that all major kinase groups and most kinase families are shared among metazoans, and many are also found in yeast, reflecting the breadth of conserved functions mediated by kinases. This ancient conservation enables cross-species analysis of function, particularly of human kinases in simpler model systems. Of 209 subfamilies, 51 are present in all four genomes, and 144 are present in all metazoans, indicating that most divergence of kinases into specific functions and families occurred during early eukaryotic and metazoan evolution. Further information on the conserved families is available at http://www.kinase.com. We focus here on the lineage-specific families and on their functional and evolutionary significance.

Fungal-specific and metazoan-specific kinases

Comparison of the *S. cerevisiae* kinome with those of worm, fly and human reveals the presence of seven yeast-specific subfamilies, containing 23 kinases (Table 2). All seven families are conserved in the distantly related fission yeast *Schizosaccharomyces pombe* [8] and accordingly mediate mainly unicellular-specific functions, including osmotic and other stress responses, cell wall signaling, cell cycle and small-molecule transport [9]. In addition to these families, nine unique yeast kinases have no close homologs in any organism.

The other 97 yeast protein kinases belong to 55 subfamilies that are shared with higher organisms. Conversely, in the common ancestor of fly, worm and human, we estimate the presence of an additional ~94 subfamilies, including two new groups (TK and TKL: tyrosine kinase and tyrosine kinase-like). These metazoan-specific families are required for the signaling functions and the sophisticated control required for development, differentiation and intercellular communication.

Kinase loss and expansion in metazoans

Nematode, insect and vertebrate lineages diverged during or before the Cambrian explosion, ~600 million years ago. There is debate as to whether insects are closer to nematode or vertebrate lineages, based both on gene sequences and body structures [10–12]; the Ecdysozoa clade groups insects and

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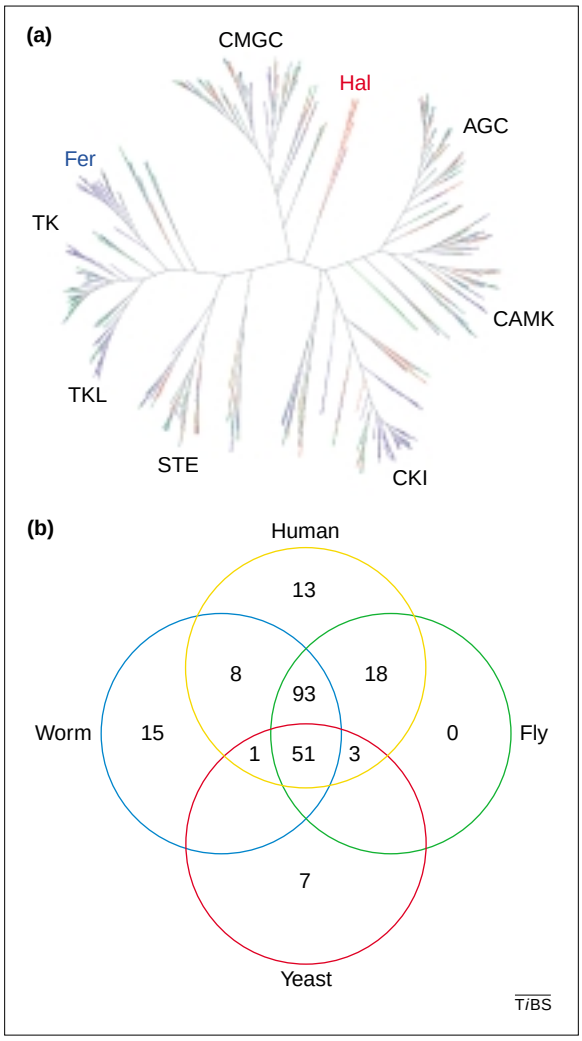

Fig. 1. (a) Hyperbolic tree representation of yeast, worm and fly protein kinase (ePK) domains. Major branches representing seven distinct protein kinase groups are labeled in black (Table 1). Yeast branches are red, worm blue, and fly green. Organism-specific expansions of various protein kinase groups are evident as mono-colored regions; the worm-specific Fer and the yeast Hal families are labeled in color. (b) Distribution of the 209 kinase subfamilies among the four genomes.

nematodes as moulting animals, whereas the more traditional coelomate clade includes both vertebrates and insects. Comparison of fly, worm and human kinomes should illuminate this early stage of metazoan evolution and show how gene loss and duplication within each lineage correlates with specific functions.

Table 1. Summary classification of protein kinases in yeast, worm and fly[a]

Group[b]	Families	Subfamilies	Yeast kinases	Worm kinases	Fly kinases
AGC	14	21	17	30	30
Atypical	12	22	15	20	16
CAMK	17	33	21	46	32
CK1	11	13	4	85	10
CMGC	8	24	21	49	33
Other[c]	37	39	38	67	45
Ste	3	13	14	25	18
TK	30	30	0	90	32
TKL	7	13	0	15	17
RGC	1	1	0	27	6
Total	140	209	130	454	239

[a]Abbreviations: CAMK, Ca^{2+}/calmodulin-dependent kinase; CK1, casein kinase 1; MAPK, mitogen-activated protein kinase; TK, tyrosine kinase; TKL, tyrosine kinase-like; RGC, receptor guanylate cyclase. For further definitions, see Ref. [6] or www.kinase.com.
[b]See http://www.kinase.com for a complete classification.
[c]The 'Other' group consists of families without strong similarities to other groups.

Despite its simpler biology, worm has almost twice as many protein kinases as fly. Almost all of that increase (203 of 216 genes) results from large expansions in certain kinase families in worm, notably the casein kinase 1 (CK1) group (85 in worm: 10 in fly), Fer (42:1), receptor guanylate cyclase (27:6), Kin-16 (16:0), Haspin (12:1), glycogen synthase kinase 3 (GSK3) (9:3), checkpoint kinase 1 (CHK1) (7:1), Ste7 (10:4), Jnk (5:1), Kin-6 (5:0), and a further 12 kinases in five worm-specific families. Little is known functionally about these expanded families, although similar expansions have been seen in non-kinase gene families and many are a result of recent genomic duplications and might contain a high proportion of pseudogenes [13–16]. Fly kinase families are more evenly spread; no family is fly-specific or has more than two kinases extra in fly than in worm. The 21 families found in fly but not worm are all present in human, but, of the 24 families found in worm but not fly, 15 are also absent from human (Fig. 1b).

Although the human kinase count is predicted to be ~500, it has only 13 families that are not found in either fly or worm, indicating that most of the large-scale divergence of kinase families had already occurred in their most recent common ancestor (G. Manning *et al.*, unpublished data). The human unique families are from the aPKs (BCR, FAST, G11, DNA-PK), Ca^{2+}/calmodulin-dependent kinases (HUNK, Trio), TKs (Axl, Lmr, Tie), TKLs (RIPK) and Other (NKF3–5) groups.

Kinase families shared by fly and human but not by worm

The 18 families found only in fly and human might encode more recent functions that developed after their putative divergence from the nematode lineage (Table 3). Some might also have been present in early metazoans but lost secondarily from the worm, as is probably the case for the three additional families found in yeast, fly and human but not worm. Within these coelomate-specific families there is a preponderance of kinases associated with immunity (nine fly genes), neurobiology (six to eight), cell cycle control (six) and morphogenesis (four), as described below.

Kinases in immunity

There are many parallels between insect and vertebrate innate immunity and inflammatory responses, including the Jak–Stat and nuclear factor κB (NF-κB) pathways and kinase families involved in human lymphocyte function.

In vertebrates, cytoplasmic Jak tyrosine kinases couple cytokine receptors to Stat transcription factors, to mediate cytokine signaling in hemopoiesis and immunity. In the fly, a single Jak (hopscotch), Stat (marelle) and cytokine-like receptor (domeless) recapitulate this pathway, which controls immune cell proliferation as well as multiple steps in tracheal development, embryonic patterning, oogenesis, testicular and eye development and sex

Table 2. Fungal-specific protein kinase families[a]

Group	Family	Subfamily	# yeast kinases	Functions and notes
Atypical	HistK		1	Osmotic stress response; related to bacterial histidine kinases
CAMK	CAMKL	Kin1	2	Cell surface localized; cell polarity and cell wall maintenance in *S. pombe*
CAMK	CAMKL	Kin4	2	Cell cycle?
CAMK	CAMKL	GIN4	3	Cell wall signaling during cell cycle
Other	RAN		3	Glucose starvation response; meiosis; nuclear export control
Other	HAL		9	Stress response; small-molecule transmembrane transport
Other	CAMKK	ELM	3	Osmotic stress response; DNA replication; budding (ELM1)

[a]Abbreviations: HistK, histidine kinase; CAMK, Ca^{2+}/calmodulin-dependent kinase; CAMKL, CAMK-like; *S. pombe*, *Schizosaccharomyces pombe;* CAMKK, CAMK kinase. For further definitions, see www.kinase.com.

determination [17,18]. Multiple Stat genes are found in both worm and *Dictyostelium*, but neither has a Jak; in worms Stats may be directly activated by receptor tyrosine kinases (RTKs) [5], implying that Jaks and cytokine receptors have evolved since divergence and co-opted Stats into their pathway.

Inflammatory signaling in both fly and human is mediated by variants of the NF-κB pathway [19,20]. In the fly, the Toll pathway (Fig. 2a) responds to fungal and Gram-positive bacterial infection and to developmental cues, while the related imd pathway (Fig. 2b) responds to Gram-negative bacterial infection. The equivalent pathways in human mediate signaling downstream of interleukin 1 (IL-1) and tumor necrosis factor α (TNFα), respectively. The Toll pathway includes the receptor-associated kinase pelle (pll) and requires phosphorylation of the cactus (IκB) protein to release the NF-κB homologs Dorsal or DIF to induce anti-microbial gene transcription. It is not known if pll phosphorylates cactus directly. Comparison with the human IL1 pathway indicates that other kinases, such as Tak1 and IκB kinase (IKK), might be involved. Tak1 and IKK have multiple fly homologs (Tak1, Takl1, Takl2 and ird5, ik2 respectively), which might function redundantly in this pathway and so be missed by the genetic screens used to characterize Toll signaling. Both Tak1 and ird5 are required non-redundantly in the imd pathway, although Tak1 has not been implicated in the equivalent human TNFα response pathway. ik2, the other fly IKK, is orthologous to IKKε and tank-binding kinase 1 (TBK1), which function in little-studied variants of the pathway [21]. Toll signaling is also used to establish dorso-ventral polarity in the fly embryo, which is probably a secondary role

Table 3. Kinase families present in fly but not worm[a]

Group	Family	Subfamily	# yeast kinases	# fly kinases	# human kinases	Functions and notes
Present in fly and human						
Other	IKK		-	2	4	Immunity
TK	JakA		-	1	4	Immunity
TK	JakB		-	1	4	Immunity; non-functional second domain of Jak kinases
TK	Syk		-	1	2	Immunity; morphogenesis
TK	Tek		-	1	5	Immunity; testis morphogenesis
Other	Slob		-	2	1	Neuronal (synaptic transmission)
Ste	Ste20	NinaC	-	1	2	Neuronal (phototransduction)
TK	CCK4		-	1	1	Neuronal (pathfinding), cancer. Secondarily lost from worm
TK	Musk		-	1	1	Neuronal (synaptic transmission)
TK	Ret		-	1	1	Neuronal (neurotrophin receptor)
TK	PDGFR/VEGFR		-	2	8	Angiogenesis
TK	Sev		-	1	1	Morphogenesis
TKL	LISK	LIMK	-	1	2	Morphogenesis; neural (pathfinding)
TKL	LISK	TESK	-	1	2	Morphogenesis
Other	MOS		-	1	1	Cell cycle; meiosis
Other	TOPK		-	1	1	Cell cycle
CAMK	Trb		-	1	3	Cell cycle
CMGC	CDK	CDK10	-	1	1	Cell cycle
Present in fly, human and yeast						
Other	TTK		1	1	1	Cell Cycle; also found in *S. pombe*, *Arabidopsis*
Other	CDC7		1	2	1	Cell Cycle; also found in *S. pombe*
CAMK	CAMKL	PASK	2	1	1	Also found in *S. pombe*

[a]Abbreviations: IKK, IκB kinase; TK, tyrosine kinase; CCK4, colon carcinoma kinase 4; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor; TKL, tyrosine kinase-like; LIMK, LIM domain kinase; TESK, testis-specific protein kinase; TOPK, T-cell-originated protein kinase; CAMK, Ca^{2+}/calmodulin-dependent kinase; CDK, cyclin-dependent kinase; *S. pombe*, *Schizosaccharomyces pombe;* CAMKL, CAMK-like; PASK, PAS-domain kinase. For further definitions, see www.kinase.com.

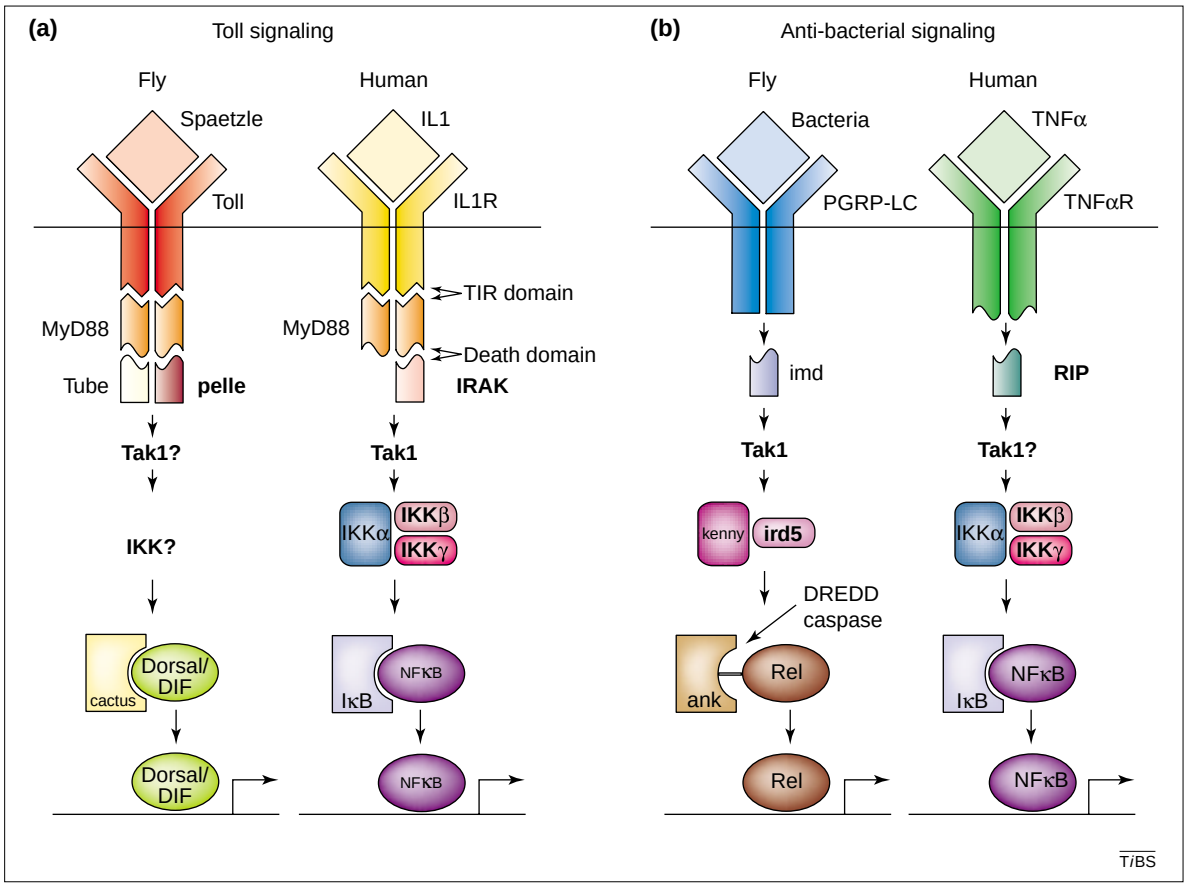

Fig. 2. Kinase signaling in innate immunity. Kinases are shown in bold. (a) Toll signaling in fly controls dorso-ventral patterning and the anti-fungal response, and is similar to IL1R and Toll signaling in human. The MyD88 adaptor binds to Toll via their TIR domains, and binds pelle and tube through death domains. This complex is required for the phosphorylation of cactus, leading to release and nuclear translocation of the NF-κB transcription factors DIF or Dorsal. In IL1 signaling, Tak1, and IKKα/β kinases mediate this effect; Tak1 and IKK homologs in the fly may have a similar function. (b) Antibacterial response in fly is controlled by the imd pathway, most similar to human TNFα signaling. The pelle-tube complex is absent, replaced by the imd adaptor and a TRADD–TRAF complex (not shown). This activates Tak1 in fly, which activates the kenny/ird5 IKK complex to phosphorylate the cactus-like ankyrin repeat region (ank) of the Relish (Rel) protein. This causes proteolytic processing by the DREDD caspase and release of the rel domain of Relish to translocate to the nucleus. The human pathway uses the imd homolog RIPK, but Tak1 has not been implicated in this pathway. Abbreviations: IL1R, interleukin 1 receptor; TIR, Toll/interleukin 1 receptor domain; IKKα/β, IκB kinase α/β. For further definitions, see Ref. [6] or www.kinase.com.

developed within the arthropod lineage. Fly has two Tak1 homologs, a MyD88 homolog (CG2078), nine toll-like receptors, 12 peptidoglycan recognition protein (PGRP) genes and a TNFα receptor-like gene, indicating that several other variants of these pathways exist [22]. The worm genome contains a single pll homolog, and two Tak1-like genes, which might be involved in Jnk activation, as well as possible homologs of Toll and cactus. Apart from Toll, the products of these genes appear to have no immune function [23], and worm lacks homologs of NF-κB and IKK. Hence both Jak–Stat and NF-κB pathways appear to have developed by adding new kinases and other genes to modify previously existing pathways.

The fly kinome also contains two cytoplasmic tyrosine kinases, shark and Btk29A, whose vertebrate homologs function in lymphocytes, a cell type with no fly analog. Shark has twin SH2 domains, flanking an array of five ankyrin repeats. Two mammalian homologs that lack the ankyrin repeats, Syk and Zap70, mediate signaling downstream of B and T cell receptors, respectively. Syk is also expressed in breast epithelial tissue, where it acts to maintain epithelial cell fate [24]. Shark is expressed in embryonic epithelia and is localized to the apical region; it functions in the process of dorsal closure. Two Syk kinases are found in the primitive metazoans *Ephydatia fluviatilis* and *Hydra vulgaris* [25,26]. In each, one gene is clearly closer to vertebrate Syks and the other to shark, including the ankyrin repeats in the *Hydra* gene. Thus, it appears that early metazoa had two Syk kinases, one of which was lost in insects, the other lost in vertebrates and both lost from nematodes. Shark is, therefore, a paralog rather than an ortholog of mammalian Syks. Btk29A is the fly ortholog of the Tec family, whose five human members are mostly associated with B and T cell function. Btk29A is best known for developmental roles such as morphogenesis of the male genitalia and oogenesis [27,28], but it is also expressed in lymph nodes and macrophages, indicating an ancestral hemopoietic expression and function.

Neuronal kinases

The increased complexity of coelomate nervous systems is reflected by the existence of several

coelomate-specific kinase families with members involved in various neural functions, including axonal pathfinding, development, vision and neuromuscular synaptic transmission.

In flies, axonal pathfinding requires the RTK off-track (otk, previously called Dtrk), which is activated by homophilic adhesion in association with the semaphorin guidance receptor PlexA [29]. Its human ortholog, CCK4, is also expressed in the nervous system and is associated with colon cancer [30]. Curiously, both otk and CCK4 are thought to be catalytically dead, as is the orthologous protein Lemon from *Hydra*, although their kinase domains are highly similar to each other, indicating strong sequence and functional conservation over hundreds of millions of years in the absence of catalytic activity [31]. Derailed (drl) is another catalytically inactive fly RTK involved in axonal guidance. Unlike otk, it does have a worm homolog (lin18) and homologs in human (ryk) and fly (doughnut and Derailed-2), all of which lack key catalytic residues [32]. Both families are thought to transduce signals by association with an active tyrosine kinase. The semaphorin signaling pathway also involves the coelomate-specific LIMK1 and center divider (cdi) cytoplasmic kinases (see below).

Among the fly neuronal kinases, Nrk is a curious hybrid. Its expression is neuron specific, but its kinase domain sequence is most similar to vertebrate MuSK, a muscle-specific agrin receptor expressed at the neuromuscular junction [33]. MuSK is an offshoot of the Trk family of neurotrophin receptors, which are otherwise absent from fly and represented only by a very divergent fragment in worm. The extracellular domain of Nrk has a kringle domain, as does the related Ror kinase family, whose members are also expressed neuronally [34]. Thus, Nrk might serve a function related to that of MuSK, Trks and Rors in vertebrates. The divergent kinase Slob also functions at the neuromuscular junction, presynaptically. Like otk and drl, it appears to be catalytically dead and probably serves as a signaling scaffold or adaptor. When phosphorylated by CaMK II, Slob binds to the Slowpoke (slo) Ca^{2+}-dependent potassium channel, serving as an adaptor for binding of the regulatory 14-3-3 ζ protein [35]. Slob has a fly paralog, CG8726, and one uncharacterized human ortholog (G. Manning *et al.*, unpublished data).

Despite their different mechanisms of vision, fly and vertebrate share several retinal-associated kinases that are absent from the eyeless worm. NinaC is an unconventional myosin that contains both kinase and calmodulin-binding domains. It is expressed in photoreceptor cells where it is involved in phototransduction and retinal structure [36,37]. Of the two vertebrate homologs, one (Myo3A) has been cloned from retina, where it is thought to fulfill a similar function [38]. Sevenless (see below) is another coelomate-specific kinase that is required for eye development. Ret RTK is expressed in the developing eye, and has probable roles in axonal guidance and proliferation [39]. Both genes have single human orthologs.

Morphogenesis kinases

LISK kinases stabilize actin cytoskeletal structures by phosphorylation and inactivation of cofilin. LISK consists of two sub-families: the LIM kinases (LIMK) and the testis-specific protein kinases (TESK). Fly LIMK1 and its human orthologs LIMK1 and LIMK2 contain PDZ and LIM domains, whereas human TESK1 and TESK2, and their fly ortholog cdi, lack these domains.

In both fly and human, LIMKs transduce signals from the small GTPases Rho, Rac and Cdc42 through intermediate kinases (Rock, Pak1 and MRCKα, respectively, in human) to the actin cytoskeleton [40,41]. Similar to otk/CCK4, human LIMK1 is part of a semaphorin signaling pathway [42] and is implicated in brain and neurite development. Human and fly TESKs have different reported functions. Human TESKs are expressed selectively and function in different subsets of sperm cells [43], whereas fly cdi is expressed in the embryonic nervous system and required for axonal pathfinding [44]. EST sequencing shows significant expression of cdi in adult testis and of TESK1 and TESK2 in neuronal tissue indicating that, although they have been studied in different tissues, they might have related functions in both neurogenesis and spermatogenesis. Worm has homologs of LISK upstream kinases and cofilin, and has small GTPases that are involved in cytoskeletal remodeling. Its lack of LISK kinases indicates that other pathways also link small GTPases to the cytoskeleton.

Fused (fu) is a fly kinase that is part of the Hedgehog (Hh) signaling pathway, which is involved in pattern formation in embryonic segmentation and imaginal disc development. Fu acts downstream of the Ptc–Smo receptor complex for Hh to activate the Ci transcription factor, in part by antagonism of the Su(fu) protein [45]. The vertebrate Hh pathway is very similar [46] and functions in development and polarity of limbs, and in organogenesis and cancer. Worm lacks both fu and Su(fu), but does contain possible homologs of other Hh pathway genes that are implicated in sex determination and determination of reproductive and sex-selective tissues; these might constitute a related pathway [47].

Fly sevenless is the receptor for the boss integral membrane protein and acts to determine the fate of the R7 photoreceptor cell. Ros is its single vertebrate ortholog, which is expressed selectively and often transiently in growing epithelia and has oncogenic transforming ability. The mouse knockout shows defects in spermatogenesis only, indicating restricted or redundant functions for this gene [48].

Cell-cycle control kinases

Four protein kinase families that are found in fly and human, but not worm, are associated with cell cycle control (Table 3); two more are also found in yeast,

Table 4. Kinase families found in human and worm but not fly or yeast[a]

Group	Family	Subfamily	# worm kinases	# human kinases	Functions and notes
AGC	SGK		1	3	Serum/glucocorticoid induced kinases; related to AKT family
Atypical	Alpha	eEF2K	1	1	Dictyostelium homolog implies secondary loss in fly
CAMK	CAMKL	MELK	1	1	Related to MARK family, present in fly
CAMK	PSK		1	2	Unknown function
CAMK	PIM		2	3	Related to PASK which is present in fly but not worm
TKL	MLK	HH498	1	1	Unknown function
TK	Trk		1	3	Fly MuSK or RORs may serve a Trk function. Worm gene is a divergent fragment
TK	Met		2	2	One worm gene is very divergent

[a]Abbreviations: eEF2K, eukaryotic elongation factor 2 kinase; CAMK, Ca^{2+}/calmodulin-dependent kinase; CAMKL, CAMK-like; TKL, tyrosine kinase-like. For further definitions, see www.kinase.com.

indicating that some of these functions have been lost in worm rather than having recently evolved. Most of these genes are known in fly only from genomic predictions. CDC7 (CG5790 in fly) and CDK10 (cdc2rk) are probably cell cycle regulatory kinases, Mos (CG8767) is a divergent kinase implicated in meiosis, and tribbles (trbl) acts as a mitotic blocker in development. TTK (CG7643 in fly) is expressed differentially during cell cycle, and T-cell-originated protein kinase (TOPK) (CG8173 in fly) is a putative p38 MAPK kinase which is differentially phosphorylated during the cell cycle and interacts with a homolog of the fly discs-large tumor suppressor in human [49].

Kinases families absent from fly

Eight kinase families have members in worm and human but not yeast or fly (Table 4); if, indeed, human is closer to fly than to worm, these families must have been lost secondarily or must be highly diverged in fly or are not yet covered by the genome sequence. The aPK eukaryotic elongation factor 2 kinase (eEF2K) is orthologous to myosin heavy chain kinases from the slime mold *Dictyostelium discoideum* and was almost certainly lost from *Drosophila*. For three other families, *Drosophila* has closely related kinases that may fulfill a similar function (Table 4).

Unusual evolution of split kinase receptors

In general, fly and worm have similar numbers in each kinase family, with expansions in human. The 'split kinase' RTKs are an exception, with unique expansions and extracellular domains found in both fly and worm. The split kinases have an insert in their catalytic domains and arrays of extracellular immunoglobulin repeats. They include the vascular endothelial-, platelet-derived- and fibroblast-growth factor receptor (VEGFR, PDGFR and FGFR, respectively) families, which function as growth and chemotactic factors in angiogenesis and organogenesis [50].

Two well-characterized FGFRs in fly function in the morphogenesis of the heart (htl) and the tracheal system (btl), a branched tubular network structurally similar to the vertebrate vasculature, both tissues that are absent from worm. Worm has a single FGFR, egl-15, which is required for sex myoblast migration and larval viability.

Worm has no members of the VEGFR or PDGFR families, but fly has two kinases, Pvr and CG3277, that are equally similar to both families and probably evolved from their common ancestor. Pvr functions along with the epidermal growth factor receptor in directed cell migration [51].

Both worm and fly also have several divergent split kinase receptors, which are weakly similar to FGFRs in their catalytic domains, but which have unique extracellular regions. One of the four fly genes, torso, determines terminal cell fate in the embryo through a Ras signaling pathway [52]. The other three fly genes are uncharacterized and have unusual extracellular regions: tie has a mucin-like domain, WSCK has a WSC putative carbohydrate-binding domain, and HD-14 has a divergent cadherin-like domain. The two worm genes are fragmentary predictions; R151.4 has no extracellular region, and Y38H6C has an extracellular region with no sequence similarity to any other gene.

The large worm-unique *kin-6* and *kin-16* families also have split tyrosine kinase domains. Some have an array of immunoglobulin repeats in their extracellular regions, others have vestigial extracellular regions [53]. The *kin-16* family includes *old-1* and *old-2*, the products of which are thought to mediate age or stress resistance. The chromosomal clustering of *kin-16* genes and their poor conservation in *Caenorhabditis briggsae* suggests that this family might be recently evolved and be specific to *C. elegans* [53], similar to other worm gene expansions [15].

Concluding remarks

Protein kinases can be classified into many families, most of which are conserved throughout metazoans, and many of which are also conserved in yeasts. The worm kinome has large expansions of several families, but has also clearly lost other families found in more primitive metazoans and even fungi. The fly also appears to have lost some kinase families but is, overall, a closer model to the human kinome and contains 21 kinase families absent from worm but

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present in human, including several involved in immunity, neural function and morphogenesis. This, and the absence of any kinases found only in fly and worm, argues for the earlier divergence of nematodes from the main metazoan lineage. Such cross-species classifications can illuminate the role of novel genes in known pathways such as immunity, and the evolutionary profile can help predict which kinds of proteins might interact with each other in conserved pathways.

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