Chapter 2

The Age of Protein Kinases

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Abstract

Major progress has been made in unravelling of regulatory mechanisms in eukaryotic cells. Modification of target protein properties by reversible phosphorylation events has been found to be one of the most prominent cellular control processes in all organisms. The phospho-status of a protein is dynamically controlled by protein kinases and counteracting phosphatases. Therefore, monitoring of kinase and phosphatase activities, identification of specific phosphorylation sites, and assessment of their functional significance are of crucial importance to understand development and homeostasis. Recent advances in the area of molecular biology and biochemistry, for instance, mass spectrometry-based phosphoproteomics or fluorescence spectroscopical methods, open new possibilities to reach an unprecidented depth and a proteome-wide understanding of phosphorylation processes in plants and other species. In addition, the growing number of model species allows now deepening evolutionary insights into signal transduction cascades and the use of kinase/phosphatase systems. Thus, this is the age where we move from an understanding of the structure and function of individual protein modules to insights how these proteins are organized into pathways and networks. In this introductory chapter, we briefly review general definitions, methodology, and current concepts of the molecular mechanisms of protein kinase function as a foundation for this methods book. We briefly review biochemistry and structural biology of kinases and provide selected examples for the role of kinases in biological systems.

Key words: Kinase, Phosphorylation, Phosphatase, Development, Cascade, Signalling, Plant

1. A Primer on Protein Kinase Biochemistry

1.1. Phosphotransfer

Kinases or phosphotransferases are *per definitionem* enzymes that catalyze the transfer of the γ-phosphate group from high-energy donor molecules such as adenosine-5′-triphosphate (ATP; sometimes guanosine-5′-triphosphate, GTP) preferentially to alcohol or hydroxy groups (OH) but also to nucleophilic centres of other functional groups of acceptor molecules, i.e. oxygen-, sulphur-, and nitrogen-containing ones (for details, see below; (1)). The process of attaching such a phosphorous-containing group is termed phosphorylation and leads to a phosphono or, in short, a

N. Dissmeyer and A. Schnittger (eds.), *Plant Kinases: Methods and Protocols*, Methods in Molecular Biology, vol. 779,
DOI 10.1007/978-1-61779-264-9_2,

phospho group in the acceptor molecule and is derived from phosphonic or phosphorous acid (H_3PO_3). This functional group is often also called a "phosphoryl" group which is not correct but regarded as acceptable (2).

Together with the eight other families such as amino- and glycosyltransferases, kinases form the "transferase group" of enzymes classified as EC 2 according to the enzyme commission (EC). One of the largest groups of kinases are protein kinases (PKs), which act on and modify the activity of specific proteins. Depending on the species, PKs represent about 2–4% of all eukaryotic genes (Table 1 and references therein; (3, 4)) and it has been estimated that approximately one-third of all proteins in animal and yeast cells are phosphorylated on at least one position (5, 6).

PKs, as phosphorous-transferring enzymes, constitute the group EC 2.7 which is further broken up with respect to the acceptor sites (Table 2): It is the hydroxy (OH), the carboxy- (COOH), or nitrogenous (nitrogen-, N-containing) group of the side chains of the basic amino acids histidine (His; H), arginine (Arg; R), and lysine (Lys; K) as well as the sulphur-containing group of cysteine (Cys; C) side chains that serve as phosphoacceptors typically located on the surface of specific target molecules or substrates. In His, it is the imidazole ($C_3H_3N_2^+$) sidechain, in Arg, a guanidinium ($CH_6N_3^+$) group, in Lys, the terminal amino (NH_2) group, and in Cys, the sulfhydryl or hydrosulphide group (SH). Besides phosphorylation on the OH-groups of Ser/Thr and Tyr that forms a phosphate ester bond (O-phosphono group or O-phosphate; Fig. 1a, b), phosphorylation of the nitrogen of His, Arg, and Lys forming a phosphoramidate linkage (N-phosphono group or N-phosphate; Fig. 1c), the sulphur of Cys forming a phosphate thioester (S-phosphono group, thio or S-phosphate), and the carboxy group of aspartate (Asp; D) and glutamate (Glu; E) forming an acid anhydride (acyl phosphate) can also occur. In eukaryotic cells, hydroxy-linked phosphorylation is by far the most studied and thio and acyl phosphates are extremely rare (7).

Analysis of the abundance of phosphorylation sites revealed that the most prominent phospho acceptors of a typical PK are the three amino acids with side chains containing hydroxy groups: Ser, Thr, and Tyr (7). Accordingly, kinases have been classified into protein serine/threonine kinases (PSTKs) and protein–tyrosine kinases (PTKs). In all organisms studied so far, protein phosphorylation does not occur to the same extent at all potential acceptor side chains; serine phosphorylation occurs much more frequently than threonine or tyrosine phosphorylation. Phosphorylation of eukaryotic proteins occurs mainly on Ser (86%) and Thr (12%) residues and to a lesser extent on Tyr (2%) (8).

Despite the fact that Tyr phosphorylation seems to be underrepresented compared to Ser/Thr phosphorylation, it appears to be very dynamic (9, 10) and crucial in building extensive networks

Table 1
Kinome sizes by organism

Common name	Scientific name	No. of kinases[a]	Percent of kinases[a]	No. of genes[a]	References
Fission yeast	*Schizosaccharomyces pombe*	119	2.4	4,929	(3, 134)
Budding yeast	*Saccharomyces cerevisiae*	130	2.1	6,275	(3, 77, 135)
Choanoflagellate	*Monosiga brevicollis*	331	3.6	9,171	(136)
Red alga	*Cyanidioschyzon merolae*	67	1.3	5,331	Kinomer
Green alga	*Ostreococcus tauri*	104	2 .6	4,003	Kinomer
Slime mold	*Dictyostelium discoideum*	285	3.1	9,226	(137)
Sea urchin	*Strongylocentrotus purpuratus*	353	1.2	28,881	(138)
Nematode	*Caenorhabditis elegans*	454	2.3	19,427	(139–141)
Fruit fly	*Drosophila melanogaster*	377	2.8	13,601	(3, 142)
Mouse	*Mus musculus*	540	1.8	29,252	(68, 143)
Rat	*Rattus norvegicus*	521	2.2	24,135	Kinomer; (144); KEGG GENOME
Human	*Homo sapiens*	518	2.0	25,667	(67), KinBase; (145)
Dog	*Canis lupus*	656	3.3	19,835	Kinomer; KEGG GENOME
Chicken	*Gallus gallus*	546	3.0	18,119	Kinomer; KEGG GENOME
Mouse-ear/thale cress	*Arabidopsis thaliana*	1,112	4.1	27,361	(70, 72, 76, 146)
Rice	*Oryza sativa*	1,637	5.8	28,236	(71), The Institute for Genomic Research (TIGR, release 3; http://www.tigr.org/) (72, 73, 78)

(continued)

Table 1
(continued)

Common name	Scientific name	No. of kinases[a]	Percent of kinases[a]	No. of genes[a]	References
Maize	*Zea mays*	2,585	12.3	20,948	MaizeGDB; (147)
Grass	*Brachypodium distachyon*	1,177	5.7	20,562	(88)
Black cottonwood	*Populus trichocarpa*	919	2.3	40,484	PoplarDB, (86); (87), KEGG GENOME
Grapevine	*Vitis vinifera*	1,485	3.8	39,423	(89)

Overall classification (43); general data about plants (4, 74, 75); genome sizes: Kimball's Biology Pages at http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/, Royal Botanic Gardens, Kew Plant DNA C-values database at http://data.kew.org/cvalues/, Animal Genome Size Database Home at http://www.genomesize.com/, dictyBase at http://dictybase.org/, DOGS – Database Of Genome Sizes at http://www.cbs.dtu.dk/databases/DOGS/, and Kyoto Encyclopaedia of Genomes and Genes (KEGG) at http://www.genome.jp

[a]The absolute numbers of both kinases and genes varies according to the source and reference

Table 2
Transferases: phosphotransferases/kinases (EC 2.7)

Class	Group	Acceptor	Examples		
2.7.1	Monophosphotransferases	Alcohol/hydroxyl /OH^-	Phosphotransferases: hexo-, gluco-, fructo-, galacto-, phosphofructo kinase; thymidine, NAD^+, glycerol, pyruvate, phosphatidylinositol 3-kinase; glucose-1,6-bisphosphate synthase		
2.7.2		Carboxyl/$COOH^-$	Phosphotransferases: phosphoglycerate and aspartate kinase		
2.7.3		Nitrogenous/N	Phosphotransferases: creatine and arginine kinase		
2.7.4		Phosphor/phosphate /PO_4^{3-}	Phosphotransferases: polyphosphate kinase; phosphomevalonate, adenylate, guanylate, and nucleoside-diphosphate kinase		
2.7.5		Intramolecular	Phosphotransferases catalyzing intramolecular transfers; mutases. Now listed as EC 5.4.2: phosphogluco-, phosphoglycerate, and bisphosphoglycerate mutase; phosphopentomutase		
2.7.6	Diphosphotransferases	Diphosphate/pyrophosphate/$P_2O_7^{4-}$	Diphosphotransferases: ribose-phosphate and thiamine diphosphokinase		
2.7.7	Nucleotidyltransferases	Nucleotidyl	Polymerase	DNA polymerase	DNA-directed DNA polymerases: DNA polymerase I, II, and III holoenzyme, reverse transcriptase (telomerase)
				RNA nucleotidyltransferase	RNA polymerase/DNA-directed RNA polymerases: RNA polymerase I, II, III, and IV, primase, RNA-dependent RNA polymerase; polynucleotide phosphorylase
			Other	Recombinase (integrase), exonucleases (Rnase PH), transposase	
2.7.8		Other substituted phosphor groups	Transferases: *N*-acetylglucosaminephosphotransferases, phosphatidylcholine synthase		

(continued)

Table 2
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Class	Group	Acceptor	Examples
2.7.9	Dikinases	Paired acceptors	Two phospho groups are transferred from a donor to two different acceptors. Phosphotransferases/dikinases: pyruvate, phosphate/water dikinases; phosphoglucan, water dikinase
2.7.10	Protein kinases	Protein, tyrosine	Protein Tyr kinases (PTKs): receptor protein–tyrosine kinase and non-specific protein–tyrosine kinase
2.7.11		Protein, serine/threonine	Protein Ser/Thr kinases (PSTKs): cAMP/cGMP-dependent protein kinases, PKC, β-adrenergic-receptor kinase, CaMK, PhK, polo, Cdk, MAPK, and MAP3K/MAPKKK/MEKK, tau, receptor PSTKs
2.7.12		Protein, tyrosine, and serine/threonine	Dual-specificity kinases (DSKs): acting both on Ser/Thr and Tyr residues; DSK, and MAP2K/MAPKK/MEK
2.7.13		Protein, histidine	Protein His kinases: pros- and tele-kinases and His kinase
2.7.99		Other	Triphosphate protein phosphotransferase

For more information, refer to the home page of the International Union of Biochemistry and Molecular Biology (IUBMB) at http://www.chem.qmul.ac.uk/iubmb/ and The Braunschweig Enzyme Database (BRENDA) – The Comprehensive Enzyme Information System http://www.brenda-enzymes.org/

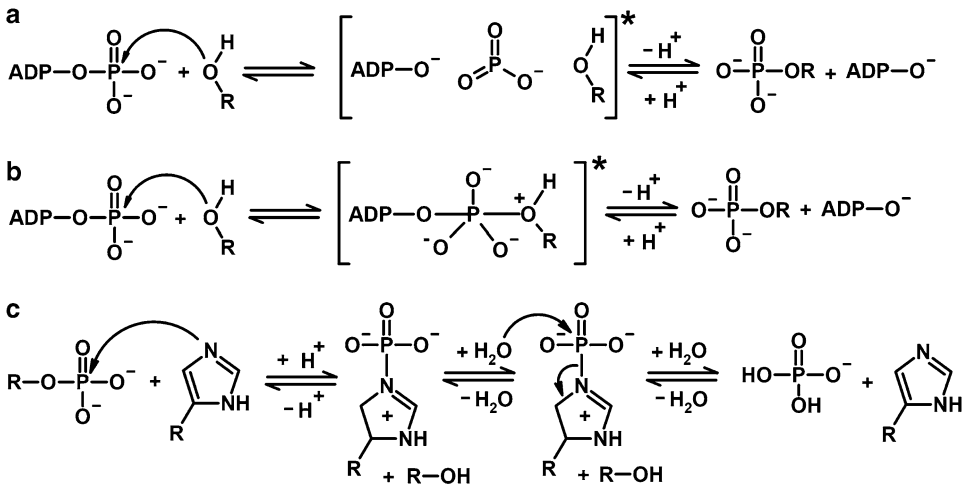

Fig. 1. Transition states for phospho or phosphoryl transfer. (**a**) Dissociative mechanism with a metaphosphate intermediate transition state. (**b**) The associative mechanism proceeds through a pentavalent transition state in which the incoming group has substantial bond formation before the leaving group has left. In the dissociative mechanism, there is accumulation of charge on the phosphoryl oxygen that links the β- and γ-phosphate groups of ATP. In the associative mechanism, there is an accumulation of charge on the peripheral oxygens of the departing γ-phosphate. Both mechanisms lead to a phosphate ester or phosphodiester bond. Transition states are shown in *brackets*. (**c**) Minimal reaction mechanism of phosphoramidate formation in phosphohistidine. (**a**) and (**b**) redrawn from ref. 166, (**c**) modified from ref. 167.

with interaction partners through their specific binding domains (11). When discussing this issue, one has to keep in mind that also the general abundance of the amino acid Tyr is much lower than that for Ser and Thr.

In plants, however, it was assumed for a long time that there is no Tyr phosphorylation at all. This was due to the lack of classic PTKs. But recent studies clearly show that Tyr phosphorylation is as extensive in plants (12–14) as it is in mammals (15, 16) and most probably performed by dual-specificity kinases (DSKs). Still, it remains open if there are Tyr-specific kinases in plants (17).

His phosphorylation has been reported in all kingdoms, and His kinases are prominent in plants and bacteria. However, N-linked phosphorylation is highly acid-labile and remained undiscovered for a long time due to the harsh experimental methods of N-terminal sequencing (Edman degradation) used in conventional protein chemical studies. Its relative contribution to the total phosphoamino acids of eukaryotic cells has now been estimated at 5–10% of total protein phosphorylation (for examples, see refs. 18, 19). However, this is up to roughly 10- to 100-fold more than tyrosine phosphorylation in eukaryotic cells, whereas Arg and Lys phosphorylation constitute less than 0.1% (20).

1.2. Biochemical and Structural Changes upon Phosphorylation

A phospho group has a pK_a of approximately 6.7 and appears predominantly dianionic at physiological pH, i.e. it forms two negative charges. Noteworthy, dianions do not occur within any of the protein-coding amino acids. The addition of a phosphoryl

group can have major impacts on the net charge of the substrate (21, 22) and can thus change the conformation of a protein (23, 24). Therefore, this often has profound effects on its functional properties such as activity, localization, and stability. The transferred phosphoryl oxygens impart a high negative charge to the target protein that on the one hand might cause a repulsion of other negative charges and on the other hand enables the formation of ion pairs with positively charged amino acid side chains, such as Arg that contains a positively charged guanidinium group with a pK_a of >12. This positively charged planar moiety interacts perfectly with the phosphoryl oxygens and is able to form multiple hydrogen bonds producing very strong bidentate interactions (25). Further interactions of the phosphoryl group predominantly involve main-chain nitrogens at the start of α-helices attracted by the positive charge of the helix dipole. The conformational and structural consequences of phosphorylation result from the sum of the newly formed and broken hydrogen bonds.

Phosphorylation can further change a protein's activation state, alter the binding efficiency for other proteins (substrates, regulators, interactors, or other kinases) and influence protein stability. For example, phosphoserine or -tyrosine residues, located within particular amino acid sequence motifs, may constitute binding sites for recognition (26). Some kinases require a hierarchical substrate phosphorylation with the first phosphorylation creating a recognition site for the kinase catalyzing the second phosphorylation and so on. Conversely, removal of a phosphate group can of course also change and reverse, for example, the catalytic activity of an enzyme and all the other aforementioned effects. Often the conformational changes result in an activation or inactivation of the protein itself which holds true both for enzymes and substrates as well, thereby regulating protein functions. For example, the conversion of an inactive to an active kinase can involve conformational changes at multiple loci in the enzyme structure, enabling the enzyme to bind substrates, orient catalytic groups, and release steric blocking of catalytic or ATP-binding sites. Intramolecular movements of the activation segment upon phosphorylation, especially of the activation loop, play key roles in kinase activation. But it is also very common that proteins are phosphorylated in order to target them to the destination site where they function in the cell, and phosphorylation sites can connect the members of a cascade with downstream events by interaction with signalling proteins that themselves contain specific phosphomotif-binding domains (11). In contrast, phosphorylation can lead to the dissociation of protein/protein complexes, e.g. in the case of Retinoblastoma protein (Rb) complexed with E2F/DP transcription factors upon phosphorylation by Cdk1 (27).

Kinases may themselves require activation by phosphorylation by another upstream kinase (e.g. cyclin-dependent kinase 1 [Cdk1]

by CDK-activating kinase 1 [Cak1]) (28) or by autophosphorylation (e.g. cAMP-dependent PK [cAPK]). In all these cases, activatory phosphorylation of the kinase introduces a structurally required double negative charge in form of the phosphono group in the activation loop. This leads to a critical re-orientation and proper alignment of catalytic residues such as aspartate and arginine that can be found in the catalytic cores or active sites of each and every eukaryotic PK (EPK). Only after having undergone these intramolecular changes, these sites together with the phosphorylated activation loop can support a nucleophilic attack of the donor phosphate (e.g. from ATP) to be finally transferred to the acceptor-site of the substrate by the kinase. However, inhibitory phosphorylation can regulate enzyme activity by initiation of conformational changes which may directly block access to the active site and/or the ATP-binding pocket, a catalytic core domain of kinases (e.g. for Cdk1; (29)), or lead to reduced substrate binding of the kinase (30). However, phosphoryl groups may also act solely as steric blocking agents without any conformational change such as in the case of isocitrate dehydrogenase (31).

Other needs for phosphorylation are when a potential phosphoacceptor site in a substrate must be brought into the proper orientation in order to be fully phosphorylated in a subsequent kinase reaction, and thus can regulate the interaction among protein partners that are required to – at least transiently – form complexes in order to function (e.g. mitogen-activated PKs (MAPKs); (32, 33)).

1.3. Kinase Structure and Function

All EPKs are structurally related to one another (34, 35); all harbour a conserved catalytic domain which consists of approximately 200–300 residues that can be further subdivided into certain characteristic domains. While the catalytic core can be separated by large amino acid insertions, the residues involved directly in catalysis are always 100% conserved. In all cases, the catalytic domain consists of two lobes where the typically small amino-terminal lobe binds and orientates ATP, and the typically large, predominantly α-helical, carboxy-terminal lobe is required for substrate binding and the initiation of the phosphoryl transfer (Fig. 2). This structural feature of EPKs is also known as the protein kinase fold. The amino-terminal domain is composed of a five-stranded β-sheet and one α-helix (the C-helix) which serves as an interaction domain and harbours a catalytic glutamate. The small lobe contains a glycine (Gly; G)-rich loop with the consensus Rossmann motif GxGxxG located in the phosphate binding loop (P-loop) which lies within the ATP-binding pocket. This structure is required for anchoring the non-transferable α- and β-phospho groups of ATP via backbone interactions and to orient the γ-phospho group for catalysis (Fig. 2). Structural analyses of the ATP-binding site and the catalytic lobes revealed that EPKs exist mainly in two conformations,

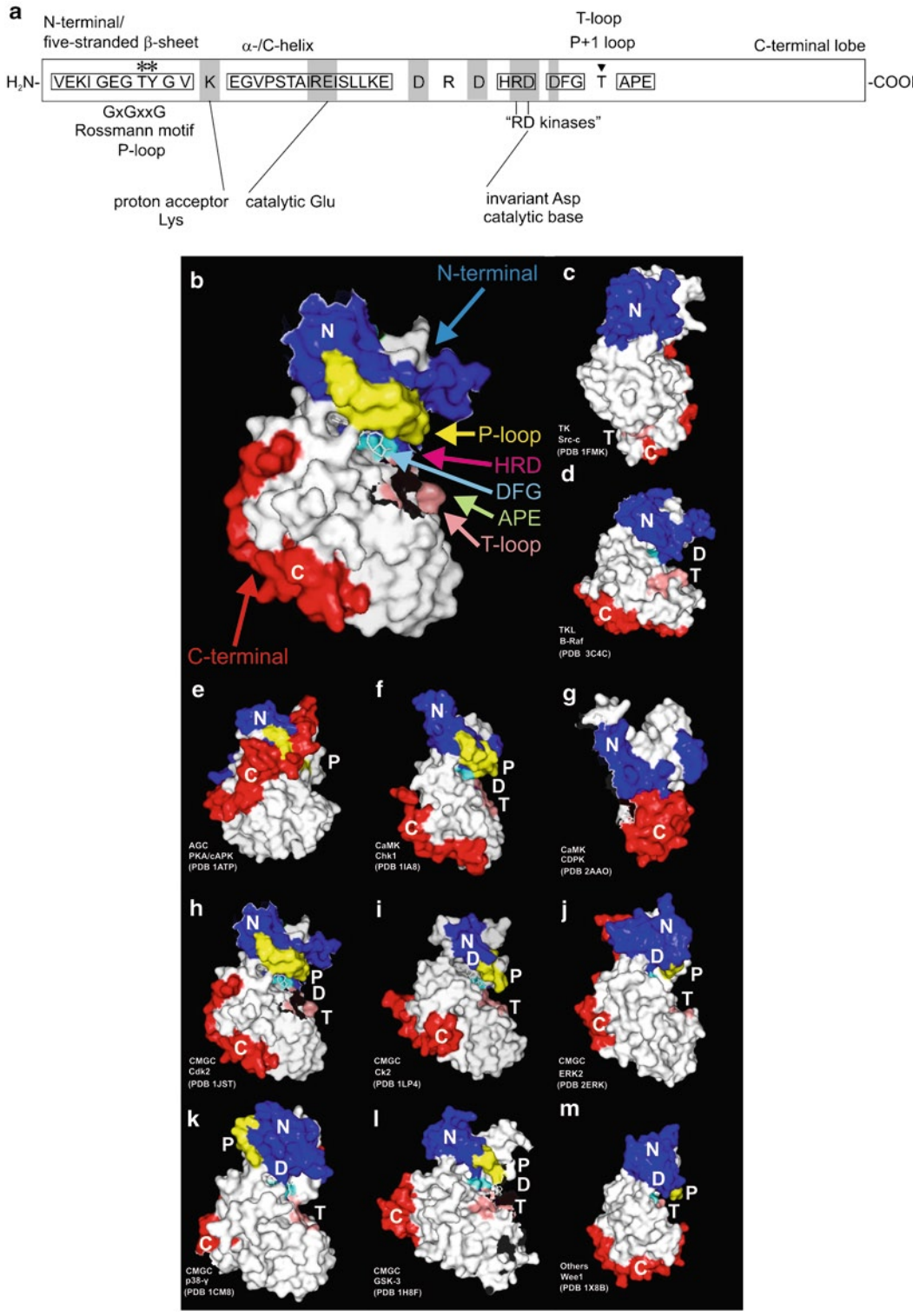

Fig. 2. Structural comparison of the catalytic cores of PEKs from different kinase families. All EPKs share highly conserved domains and catalytic residues required for their enzymatic function. (**a**) Schematic representation of catalytic EPK domains and residues, i.e. the N-terminal β-sheet with Rossmann motif in the P-loop and the α- or C-helix; the P+1 and T-loop, the HRD (eponymous with the "RD kinases"), DFG, and APE motifs in the central part of the EPKs and several highly conserved Lys, Asp, Arg, and Glu residues including the catalytic base (shaded in grey). (**b**)–(**l**) Structural representations of

i.e. open and closed (36). In the open conformation, ATP can easily access and ADP is released from the binding pocket, and in the closed form, both substrate and kinase residues are brought into proper orientation to enable catalysis. A closer look at the ATP-binding site demonstrated that five further and much more distinct pockets can be exploited for binding of chemical inhibitors by interaction with specific functional groups (37). These pockets are those utilized for adenine- and ribose-binding, the so-called p38 or gatekeeper pocket, the lysine or hydrophobic pocket below the adenine and the type II inhibitor pocket between the C-helix and the activation segment (38). The structural and atomic mechanistics of regulation of kinase activation/inactivation via protein phosphorylation are resolved on an atomic level and are reviewed by Johnson (39). Many inhibitor studies involve chemical compounds capable of specifically targeting the ATP-binding pocket of a specific kinase, which is so highly conserved throughout this enzyme family.

EPKs share a conserved catalytic domain containing likewise highly conserved lysine and aspartate residues such as Lys30, Asp125, or Asp143 (numbering refers to an approximate location within most EPK sequences, for more details see refs. 35, 40–42). The sequences of these cores serve to determine the relationships between kinases (43) and, interestingly, catalytic residues and domains of various kinases have been evidenced to adopt strikingly similar structures in their active conformation (40).

In human cyclin-dependent kinase 2 (Cdk2), the phosphate contacts three arginine residues in the C-helix (Arg50), in the catalytic strand (Arg126), and in the start of the activation segment (Arg150). In Cdks, the C-helix is also known as the Cyclin-binding PSTAIRE domain and represents a vital element of the kinase core

Fig. 2. (continued) the kinase folds of entire or catalytic regions of 11 representative members of the six major kinase families AGC, CaMK (two examples shown), CMGC (five examples shown), conventional PTK, TKL, and others. (**b**) Indicated are the small and large lobes with the N-terminal uppermost and C-terminal lowermost in the cartoon. (**c**) TK: human proto-oncogene PTK Src-c (PDB ID: 1FMK; (168)). Src has an uncommon T-loop-like structure which is significantly reduced in length and missing critical elements, and is located at the backside of the kinase fold. (**d**) TKL: human B-Raf proto-oncogene PSTK in complex with an inhibitor (PDB ID: 3C4C; (169)). Raf has an uncommon T-loop-like structure which is significantly reduced and lacking a clear P-loop. (**e**) AGC: catalytic subunit of human PKA/cAPK complexed with a peptide inhibitor (PDB ID: 1ATP; (170)). The P-loop-like structure is located at the backside of the kinase fold. (**f**, **g**) CaMK. (**f**) Human cell cycle checkpoint kinase (Chk1; PDB ID: 1IA8; (171)). (**g**) Regulatory apparatus of CDPK from *Arabidopsis thaliana* (PDB ID: 2AAO; (172)). This structure is very small and only a fragment. (**h–l**) CMGC class. (**h**) T-loop-phosphorylated human Cdk2 bound to cyclin A (cyclin moiety not shown, PDB ID: 1JST; (28)). (**i**) Binary complex of the catalytic subunit of maize CK2α (PDB ID: 1LP4; (173)). (**j**) Phosphorylated human MAPK ERK2 (PDB ID: 2ERK; (174)). (**k**) Phosphorylated human MAPK p38-γ (PDB ID: 1CM8; (175)). The P-loop-like structure is located at the backside of the kinase fold. (**l**) Human GSK-3β (PDB ID: 1H8F; (176)). (**m**) Others: kinase domain of human Wee1 complexed with an inhibitor (PDB ID: 1X8B; (177)). The P- and T-loops and conserved motifs such as active clefts, and catalytically active sites or their equivalent positions in the sequence are indicated by *shading and labels* as follows. *P* ATP-binding or P-loop, *T* activation segment or T-loop, *D* DFG motif. Open gaps in the visualized structure result from the masking of subunits and/or other binding partners within the crystal. The structures were derived from the RCSB Protein Data Bank (PDB) and Pymol (178) used for visualization. This figure is displayed in greyscale in the print edition but available in color in the electronic version.

involved in peptide-substrate recognition together with the "activation segment." In EPKs, this sequence starts with the conserved region extending from the DFG (Asp-Phe-Gly) to the APE (Ala-Pro-Glu) motif that swings down upon activatory phosphorylation to allow access to the catalytic cleft (44–46). The activation segment usually has a size of roughly 20–40 amino acids and includes, from the N- to C-terminus, secondary elements serving as Mg^{2+}-binding loop containing the DFG motif, β9, an activation loop (T-loop) and a P + 1 loop that includes the APE motif. In many but not all EPKs, a highly conserved threonine residue plays a pivotal role in functional activation of the activation loop, therefore, named the *T-loop*. In cAPK, this is a Thr-197 residue beside another autophosphorylation site, i.e. in Cdks a threonin close to the residue 160.

The so-called "invariant aspartate" (Asp166 in cAPK) within the HRDLKxxN motif (in short: HRD) of the catalytic loop functions as the "catalytic base" and "proton trap," most likely by activating the incoming substrate hydroxy group (Fig. 2). Most PSTKs and all TPKs belong to the group of RD kinases (47) which are defined as those kinases in which the conserved catalytic aspartate is preceded by an arginine residue. The arginine builds up ionic interactions with a phosphate or a carboxylate group (see above). Most, but not all RD kinases are activated by activation loop phosphorylation; these always contain an RD motif at the invariant aspartate (Table 3). Crystallography revealed that a cluster of basic residues, partially originating from the RD motif, is involved in charge neutralization of the phosphorylated amino acid of the activation loop. The dianionic phosphoryl group of such a phosphothreonine functions in stabilization of the previously mentioned positively charged cluster of Arg, Lys, and His side chains and directs the orientation of the catalytic base aspartate by producing the required electrostatic environment (35).

Interestingly, phosphorylase kinase (PhK) has a negative charge through the presence of the glutamate side chain and protein kinase CK1 accomplishes charge neutralization of the catalytic arginine in the HRD motif by binding a sulphate (SO_4^{2-}) dianion in close proximity both replacing a phosphoamino acid dianion. In CK2α, the catalytic subunit of the holoenzyme protein kinase CK2, the activation segment is unphosphorylated. Under crystallization conditions, a sulphate anion occupies the activation segment and marks the site that recognizes the acidic side chain in CK2 substrates at P + 3 (48).

The catalytic base is possibly involved in forming an alcoholate or phenolate anion with the hydroxy rest coming from a serine, threonine, or a tyrosine that is going to be phosphorylated. This anion then further attacks the γ-phospho group of ATP and results in phospho transfer. The activation loop undergoes enormous conformational changes upon phosphorylation and virtually

Table 3
RD kinases and EPKs dependent and independent on phosphorylation in the activation segment

Phosphorylated activation segment	Unphosphorylated activation segment
RD kinases	*RD kinases*
Single phospho-site	CaMK family: PhK, Chk1, CaMII
AGC family: PKA/cAPK[a], PKB, PKC	CK1 family: CK1
CMGC family: PSTAIRE-Cdks, Cak/Cdk7, GSK-3β (phosphorylation at secondary site[b])	CMGC family: CK2α, some non-PSTAIRE-Cdks (human Cdk5/6)
TK family: VEGFR and PDGFR TKs, Src family members[a]	CaMK family: CaMKI
TKL family: Raf	TK family: EGFR TK
STE family: MAPKK/MEK1	
Dual phospho-site	*Non-RD kinases*
AGC family: Aurora A	CaMK family: SKY1, MLCK
CMGC family: p38/MAPK/ERK2	CMGC family: DAPK
TK family: IRAK	Others: Wee1

RD kinases: the conserved catalytic aspartate is preceded by an arginine residue
Details are taken from refs. 35, 41, 148
[a] Autophosphorylation
[b] In all the phosphorylated kinases, phosphorylation occurs at the "primary site," if not mentioned

leads to a switch from an inactive to an active form of the kinase. In many cases, an unphosphorylated activation loop serves as a blocking structure, sterically hindering both ATP and the substrate from entering the catalytic cleft and distorting the Mg^{2+}-anchor of the DFG motif (41). Also unphosphorylated kinase with a deformed activation segment might still bind to ATP (49). However, conformational plasticity of switching EPK activation and deactivation is one of the major reasons for the rapid response of kinase cascades.

The invariant aspartate residue of the DFG motif chelates the Mg^{2+} and helps to position the phosphate for transfer (40, 50). Moreover, the phenylalanine serves as an intramolecular binding interface for the C-helix and this region often contains further conserved phosphoacceptor sites such as the signature T-x-Y of MAPKs and those of MAPK kinases (MAPKKs/MAP2Ks) and Cdks (51). Phosphorylation of serine/threonine and/or tyrosine residues in these segments has the capacity to modify the overall fold between the DFG and APE motifs. This, in turn, is a key event necessary for the correct positioning of the two kinase lobes to form an active conformation ready for catalysis (40).

For successful phosphoryl transfer, kinases require on the one hand consensus target amino acid sequences at the phosphoacceptor site. For Cdk1, this is K/RS/TPxK/Rx with S/TP being the minimal motif (52, 53). Substrate recognition, on the other hand, is mediated through the primary sequence surrounding the phosphorylatable residues in the target sequence, as well as through other docking sites further apart in the substrate's structure (54). Cdks, for example, are proline (Pro; P)-directed kinases, i.e. their its consensus target phosphorylation sites must not only contain a Pro residue but also have to be positioned at a very specific site immediately after the S/T residue to allow modification. As an example from the CMGC kinases, in human Cdk2, a valine (Val; V)-rich segment of the highly preferred phosphoacceptor site in the activation loop 160-TxxVVTL-166 is part of the P + 1 loop and contributes to an unusual left-handed conformation. Here, the side chain is recognized C-terminally of the phosphoacceptor site. This results in a tight association with two main-chain carbonyl oxygen atoms of another conserved Arg and forms a pocket that solely accommodates a Pro residue. Any other amino acid side chain containing a peptide amino group would need a supplementary hydrogen-binding partner in order to be properly accommodated (55, 56).

The activation segment of kinases is also known to literally form "assembly platforms" whereby catalytic residues of the kinase and substrate phosphoacceptors can approach in the closest proximity for a successful nucleophilic phosphoryl transfer (40, 46).

With some of the early crystal structures of human EPKs, such as for Cdk2 and MAPK, it was shown that the activation loop reduces access to the substrate site (Cdk2 (57) and MAPK (58)). However, with other EPKs, e.g. PKA (protein kinase A/cAMP-dependent PK/cAPK), a different role was proposed. Dephospho- and phosphomimetic substitutions of the activation loop Thr showed little change in peptide-substrate affinity, highlighting a more crucial role of phosphorylation at this residue in catalysis than that in substrate recruitment (59). Contrarily, bimolecular fluorescence complementation (BiFC) experiments demonstrated that hypomorphic alleles and dephospho variants with modified T-loops of *Arabidopsis thaliana* CDKA;1 displayed reduced binding to *bona fide* substrates (60, 61) (see also Chapter 14 in this book).

Some eukaryotic kinases, such as Cdks, appear to contain little more than the PK catalytic core and are almost fully inactive as monomer (62). In addition, EPKs exhibit to some extent variability in parts of the kinase domain and may contain additional domains, additional subunits, divergent intramolecular regulation mechanisms, or all of these. Thus, EPKs can be controlled through various means such as by binding to additional subunits (cyclin, Cdk subunit), domains (cyclin, SH2 and SH3, 14-3-3; (63, 64)),

ligands (cyclic adenosine monophosphate (cAMP), Ca^{2+}/calmodulin, diacyl glyerol), by controlling the abundance and/or stability of these factors (cyclin degradation by the anaphase-promoting complex/cyclosome (APC/C)), by inhibitor proteins, by phosphorylation/dephosphorylation, or via subcellular localization.

Outside their catalytic domain, PKs possess a wide diversity of recognition/interaction domains, including those for dimerization, (auto)phosphorylation, docking, acylation (membrane targeting), and recruitment (membrane, nucleus, cytosol), but also for rapid degradation (for instance, a PEST domain, a motif consisting of a hydrophilic stretch of at least 12 amino acids in length with a high local concentration of the amino acids Pro, Asp, or Glu, and Ser or Thr; PEST domains critically reduce the halflife of proteins).

Phosphorylation is a sub-stoichiometric process. This means that not the entire pool of a certain substrate protein is phosphorylated at the same time. Consequently, both quantification and even detection can be extremely difficult for low abundance phosphorylation sites. Besides that, protein phosphorylation is very abundant in multicellular eukaryotes containing a high number of kinases (Table 1) that target a multitude of substrates. This enzyme–substrate interaction can be either specific in a one-to-one fashion or, which is very common, in a much more complex way where one protein is substrate of multiple kinases with different consensus and target sites. However, it is also common that one site in one substrate is operated by a number of distinct kinases (65).

2. Kinase Classification and Abundance

At the moment, more than 17,000 kinases have been described overall, including more than 10,000 protein kinases, the class of enzymes with other proteins as a target (66). Thus, PKs in general are one of the largest families of kinases, and indeed one of the largest of all protein families, and are highly conserved across species. With as many as 518 in humans and 540 putative members in mice, PKs constitute the largest single enzyme family in mammalian genomes (http://kinase.com/, (67, 68)), and are also highly abundant in other model species (Table 1). In yeast, *Drosophila*, mammals, and *Arabidopsis*, it has been predicted that PKs phosphorylate 30% of cellular proteins (69). Plants, for example, *Arabidopsis* or rice, have about twice the number of PKs found in mammals, i.e. more than 1,000, which is a significantly higher number of genes than in any of the aforementioned organisms (Fig. 4, Table 5; (67, 70–73)). The PlantsP database already holds 1,112 (as of July 2010) entries for *Arabidopsis* kinases and in this species, protein phosphorylation utilizes more than 5% of the genes: 4.5% encode PKs, 0.5% encode protein phosphatases, and

the PSTKs themselves represent about 4% of the entire *Arabidopsis* genome, i.e. almost 1,000 different enzymes (74, 75). The high abundance of PSTKs seems to be limited to multicellular eukaryotes and if one takes into account that approximately 30% of the proteome is phoshorylated, each of the thousand plant kinases should be responsible for modification of seven proteins on average. This is probably an underestimation since many proteins are regulated by multisite phosphorylation, closely related PKs might overlap in their specificities, some PKs are highly specific for one or a few substrates only (e.g. MAP2K), and many phosphorylation sites are known to have no function (76).

The EPK superfamily can be broadly split into two groups according to their molecular phylogeny: conventional and atypical PKs (APKs). The conventional group is the largest and its members have further been sub-classified, first into five (43), then into seven (3), and finally into eight major phylogenetic groups according to sequence similarity between catalytic and accessory domains, and their mode of regulation (Table 4). The eight conventional EPK groups are: (1) AGC, (2) CaMK, (3) CMGC, (4) CK1, (5) RGC, (6) STE, (7) conventional PTK, and (8) TKL ((77); for abbreviations, see Table 4 and Appendix). CMGC, CaMK, AGC, and STE belong to the large paraphyletic clade of non-receptor PSTKs. The *Arabidopsis* and *Oryza sativa* (rice) kinomes contain members of six of these, but lack members of the Tyr kinase group. A total of 75% of all rice kinases are TKLs (Tyr kinase-like PKs), which includes the large Interleukin-1 receptor associated kinase (IRAK) family and both receptor and cytoplasmic TKs (78).

The Atypical PKs (APKs) group is composed of a small set of EPKs whose kinase domains do not share significant sequence similarity with conventional EPKs. APKs have been sorted into four groups having PK activity: (1) alpha, (2) phosphatidyl inositol 3′ kinase-related kinase (PIKK), (3) pyruvate dehydrogenase kinase (PDHK), and (4) RIO (right open reading frame; Table 4). Sequence similarity networks are useful tools for exploration of the kinase superfamily and visually display related kinases in proper relation (Fig. 3; (79)).

Some *Arabidopsis* PSTKs do not fall into either category, including the Raf ("rapidly growing fibrosarcoma or rat fibrosarcoma") or the receptor-like kinase (RLK) families, but instead constitute small clusters such as the Cdk-like kinases (CKL/CLK)/LAMMER clade (named after a short conserved stretch of Leu-Ala-Met-Met-Glu-Arg residues), CK1, and NIMA (Never in mitosis A)/NIMA-related kinase (NEK), or represent orphan sequences such as Cdk-activating kinase 1 (Cak1), phosphatidylinositol-4-phosphate 5' kinase (PPK1) and TOUSLED. The modules of the MAPK cascade MAPK (MPK) > MAP2K (MKK/MEK) > MAP3K (MEKK) > MAP4K all belong to the CMGC family (for MAPK nomenclature, please see Note 1).

Table 4
Eukaryotic protein kinase classification[a]

Group	Definition	Examples
Conventional EPKs		
AGC	The name of the AGC kinases is derived from the member as follows: cyclic nucleotide-dependent family (PKA/cAPK and PKG), the "PKC" family, PKB/Akt/RAC-alpha PSTK, βARK, the ribosomal S6 kinase family, and other close relatives. These kinases have a strong preference for phosphorylation of Ser/Thr residues that are located in a consensus sequence containing the basic amino acids Lys and Arg	PKA/cAPK (PKACα), PKG, PKC, Akt, MAST, MASTL, PVPK
CaMK	The members of the CaMK group take their name from Ca^{2+}/CaM-regulated kinases and structurally related families including CaMK and SNF1/AMP-activated PKs. Most of the members of this group exhibit activation by the binding of Ca^{2+} or CaM to a small C-terminal domain in close proximity to the catalytic core. These kinases tend to be directed towards substrates containing basic residues. Plant CDPKs contain an intrinsic CaM-like domain allowing these enzymes to self-activate in the presence of Ca^{2+}	CaMK, CaMK-like, CDPK, MAPK-associated PK, MLCK, TRIBBLES, KIN1/SNF1/Nim1, and AMPK, EF2K, PhK
CMGC	This group is named after the member families of Cdks and MAPKs/ERK, SHAGGY/GSK-3β, Prk, and the Cdc2-like kinases (CLK/CKL or LAMMER) to which the CK2 family also belongs to. The CMGC kinases are an essential and typically large group of kinases found in all eukaryotes. CMGC kinases display unique features such as the "CMGD-arginine" which is located right before the APE motif (i.e. "RAPE") near the substrate phosphorylation site in the activation loop. This Arg plays a role in substrate recognition and kinase activation. The so-called "CMGC-insert region" in the C-terminal lobe is a CMGC family specific docking region to anchor proteins. This region can be found between the APE motif and the C-terminus and is typical for all CMGC kinases. It is also called the "CDK insert" region as Cak binds here for T-loop activation by phosphorylation [41, 149]	Cdk1, MAPK/ERK, GSK-3β/SHAGGY, CLK/CKL, CK2α, SRPK, DYRK
CK1	The protein kinase CK1 (formerly called Casein Kinase 1) group represents a typically small but essential group of EPKs found in all eukaryotes	CK1, Tau tubulin kinase
RGC	The members of the small group of receptor guanylate cyclases (RGCs) are similar in sequence to the metazoan Tyr kinases which are lacking in plants, fungi, and protists. Their properties are distinct from other known EPKs; the RGC kinase domains appear to be all but catalytically inactive. It is thought that this group evolved late in the expansion of the EPK superfamily	Photoreceptor membrane guanylate cyclase
STE	The STE group includes homologs of the *S. cerevisiae* sterile kinases sterile 7, sterile 11, and sterile 20. This group contains the MAPK cascade kinases that act upstream of MAPKs of the CMGC group, where a MAPK is phosphorylated and activated by a MAP2K/MAPKK/MEK, which itself is activated by a MAP3K/MAPKKK/MEKK. The family of Raf-related MAP4K/MAPKKKK is structurally distinct from the Ste20 family, and is contained in the TKL group	Ste7-like (MAP2K/MAPKK/MEK, NIMA/NEK and NEK-like), Ste11-like (MAP3K/MAPKKK/MEKK, Cdc7), Ste20-like (MAP4K/MAPKKKK)

(continued)

Table 4 (continued)

Group	Definition	Examples
TK	The Tyr kinase (TK) group contains conventional phosphotyrosine kinases (PTKs). The active members specifically phosphorylate tyrosine residues of proteins, are distinct from DSKs which also phosphorylate Ser/Thr and are scattered within other kinase groups. TKs are found in metazoans but not in single celled fungi and play important roles in intracellular signalling cascades. Proteins phosphotyrosine were first characterized in plants and algae	EGFR, Jak, VEGFR, Src, LRR-TK, Abelson kinase (c-Abl), LCK
TKL	The Tyr kinase-like (TKL) group is the most diverse of all groups, is closely related to TKs, and is integrated within important signalling pathways. However, TKLs are in fact serine/threonine protein kinases and present in metazoans and plants but virtually absent from fungi. TKLs represent the largest group of EPKs in land plants, whe re they often constitute up to 80% of the kinome	LRR kinases, MLKs, TGFβR1, Raf, IRAK, RLK/PELLE family (RTK-like, Pti1-like, WAK-like)
Atypical EPKs		
Alpha	The Alpha group of kinases is a small and only recently discovered group of atypical EPKs (APKs). In mammals, channel-kinases are members of Alpha APKs. Alpha kinases contain both a fold similar to EPKs and key amino acid residues known to be important for catalysis from EPKs	EEF2K, Dictyostelium MHCK, two channel-kinases, TRPMs ion channel-kinases
PIKK	The members of the small phosphatidyl inositol 3′ kinase-related kinase (PIKK) family are involved in signalling of DNA damage and cell growth. PIKK kinases have a high molecular weight and in mammals, five of the six members act as PSTKs	PIKK, ATM, ATR, mTOR, DNA-PK, SMG-1
PDHK	The pyruvate dehydrogenase kinases (PDHKs) are a small and ubiquitous group of APKs; five members were identified in humans. PDHKs phosphorylate a subunit of the pyruvate dehydrogenase multienzyme complex and this plays an important role in oxidative metabolism, i.e. in controlling glucose and lipid oxidation	PDHK, PKAK1
RIO	The RIO ("right open reading frame") group is named after the divergently transcribed founding member which was one of the two adjacent genes. RIO is a small group of essential eukaryotic APKs. They share the overall EPK fold, however, distinct substrate-binding domains were not yet discovered and the mode of ATP-binding also seems to be different from other kinases. Some RIO kinases autophosphorylate	Rio1-family and Rio2
Others	This group contains several kinases and families that do not fit within any of the other main kinase groups	Wee1, Myt1/Mik1, Caks, CAKAK, Aurora, Mos, CaMKK, NIMA/NEK, TOUSLED, PPK1

Further references available at http://kinase.com/, http://www.compbio.dundee.ac.uk/kinomer/, and http://www.bio.unipd.it/molbinfo/PTKtable.html

[a]For abbreviations, see this table and Appendix

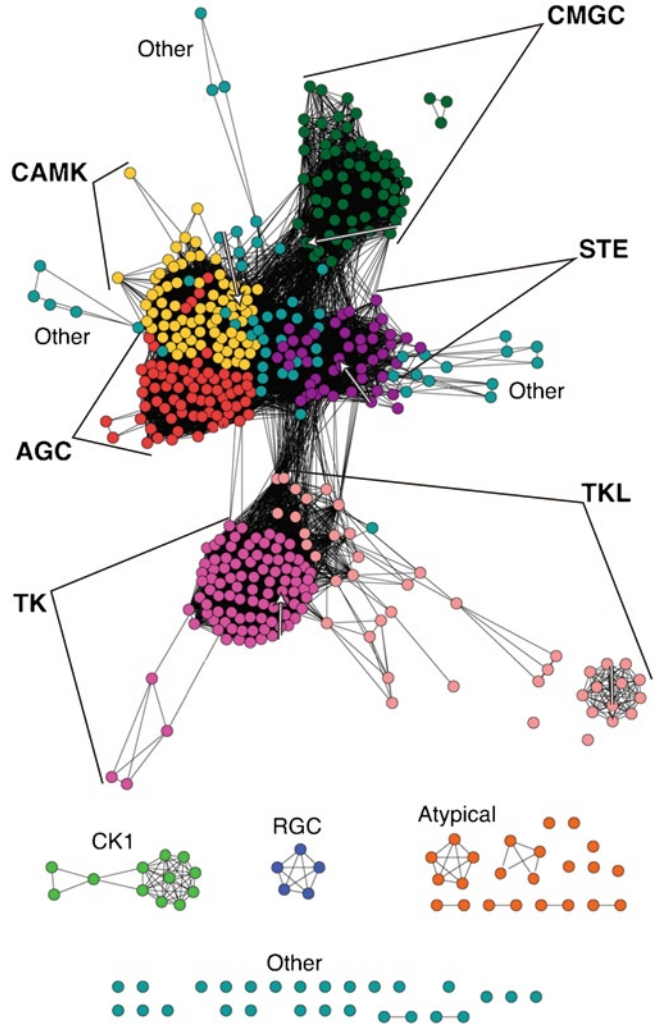

Fig. 3. A sequence similarity network visualizing the human kinome by kinase class. The network is composed of 513 human kinase domains and results from the analysis of sequences of a published phylogenetic tree (3) generated from alignments using pairwise BLAST E-values. Such networks visualize functional trends across protein superfamilies based on sequence similarity. Accordingly, functional themes and outliers can be identified. In the presented network, each kinase class appears in different greyscales according to the kinase classification listed in Table 4. This network highlights the relationship of kinase classes as well as sequence similarities and conservation. Modified and reprinted with permission from The Public Library of Science (PLoS) according to the Creative Commons Attribution License (CCAL) (79). For abbreviations, see Table 4 and Appendix. This figure is displayed in greyscale in the print edition but available in color in the electronic version.

The first plant protein kinase sequences were reported in 1989, and 10 years later more than 500 had been identified, including 175 in *Arabidopsis* (80). Nowadays, it is known that some of the *Arabidopsis* kinase families are specific to or largely expanded in the plant genomes, such as the approximately 600 members of the RLKs, the 30 members related to Cdks, the 34 CDPKs, 8 CRKs, 2 phosphoenolpyruvate carboxylase kinases (PPCKs), 2

PPCK-related kinases (PEPRK), 39 AGC members, the 25 CBL-interacting PSTKs, 16 His kinases, the RKL gene family which counts 307 members, the 38 Snf1-related kinases (SnRKs), the 123 genes coding for putative MAPK signalling components (including 20 MAPKs, 10 MAPKKs, 80 MAPKKKs, 10 MAPKKKKs), and the 29 CLK/CKL or LAMMER kinases (TAIR and PlantsP database; (74, 75, 81–85)).

In the wild grass *Brachypodium distachyon*, protein kinase gene families consist of 140 kinase subfamilies with 1,177 predicted members of which 904 are PSTKs. Comparison of the kinomes of rice (1,637 members, Rice Kinase Database), *Arabidopsis* (1,112 members, KinBase), and poplar (black cottonwood, *Populus trichocarpa*; 919 results for "kinase" in PopulusDB in July 2010 (86, 87)) to the *Brachypodium* kinome (a total of 1,440 PKs for annotated PSTKs and TKs/TKLs, with DSKs counted in both groups (88)) demonstrates similar composition to rice but with fewer members. Both rice and *Brachypodium* encode the same kinase subfamilies of a very similar size, with the exception of eight RLK subfamilies. These subfamilies account for nearly all (252 of 268) of the total difference in kinome size. The greatest differences were found among the non-RD kinase subclass, predicted to encode pattern recognition receptors.

In another example from the plant kingdom, 1,485 protein kinase-like (PKL) genes were annotated in grapevine (*Vitis vinifera*); with 2.3% of PKL genes found within the top 50 InterPro hits in the filtered gene set. 1,312 PKLs were predicted to contain TPK and 1,271 to have PSTK activity which could be interpreted as a plant-specific, very broad functional assignment as DSKs (89).

Remarkably, the rice kinome, for example, contains – depending on the reference source – 31 to 47% more kinases than *Arabidopsis* and is three times larger than the human kinome (Fig. 4, Table 5; (70–72, 90–92)).

It is important to note that all of the numbers quoted above are approximate because of the presence of genes encoding atypical kinases and the frequent inclusion of catalytically inactive members where usually highly conserved core residues got substituted. Because kinase family structures are not well defined in plants, kinases are assigned to subfamilies based on putative function.

2.1. Kinome Differences Between Animals and Plants

After the completion of sequencing of the *Arabidopsis* genome (70), it became apparent that plant genomes, e.g. *Arabidopsis* (~4%), rice (~6%), and maize (~12%), encode higher percentages of PK-coding genes than yeast (~2%), *Caenorhabditis elegans* (~2%), *Drosophila* (~3%), and humans (~2%; Tables 1 and 5; Fig. 4).

Comparative analyses suggest that plants have evolved both their own signalling molecules and signal transduction pathways. One supported idea is evolutionary combination or shuffling of novel sequences; however, conserved motifs are apparent in the

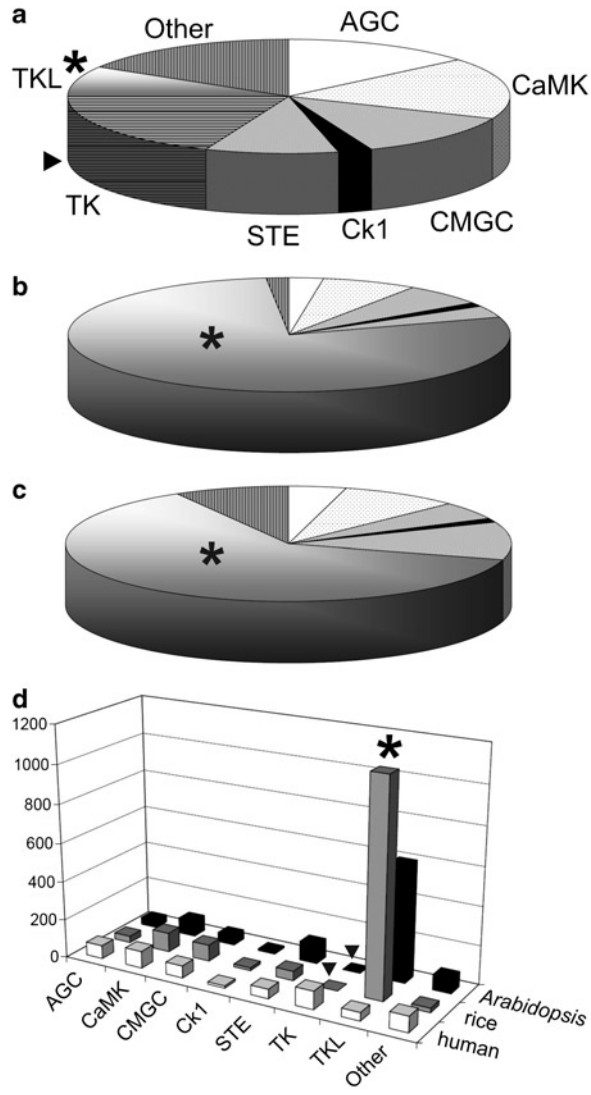

Fig. 4. Pie chart of human, rice, and *Arabidopsis* kinases. Distribution of kinases within the eight main kinase groups: AGC, CaMK, CMGC, CK1, STE, TK, TKL, and others according to Table 5. (**a**) Human, (**b**) rice, and (**c**) *Arabidopsis* kinomes. (**d**) Absolute numbers of aforementioned major kinase groups per kinome; the number of genes is shown on the ***y*** axis, subfamilies are listed along the ***x*** axis. Remarkably, the TK group (*arrowhead* in (**a**) and (**d**)) is missing entirely in both plant species (**b**, **c**). However, the TKL family (*asterisk*) is highly overrepresented in plants (**a**–**d**). Data from KinBase, The *Arabidopsis* Information Resource (TAIR), and Rice Kinase Database (RKD).

high number of plant paralogues of PSTKs as a result of gene duplications. Furthermore, plant-specific modules of ligand reception and signal transduction cascades have developed. A good example is the machinery involved in ethylene reception, which perceives the gaseous phytohormone by a two-component His kinase and further transmits the signal by MAPK cascade elements, see also step 1 of Subheading 1.3. Thus, gene duplication has radically

Table 5
List of human, rice, and *Arabidopsis* kinases by group[a]

	Human	Rice	Arabidopsis
AGC	69	35	39
CaMK	88	100	83
CMGC	63	85	50
CK1	12	15	10
STE	49	49	100
TK	94	0	0
TKL	43	1,104	600
Other	82	23	79

[a]Graphical illustration in Fig. 4

influenced the structure, organization, and function of plant PK genes and cascades leading to novel properties (93).

Genomics has also revealed that animal genomes contain PSTKs, PTKs, and His PKs, whereas in plants, PSTKs and His PKs are present exclusively. In *Arabidopsis*, most PKs are PSTKs. Plants share histidine kinases with prokaryotes and yeast (94). A couple of PK families are specific to plants, for example, the family of RLKs, absent in yeast, which play a very important role in intercellular signalling in plants. In *Arabidopsis*, the 4% of PSTKs are split up into 2.6% RLKs, 1.2% non-RLKs, and 0.2% Rafs.

Although plant genomes encode many of the animal-type kinases and hundreds of RLKs, they lack classical PTKs. The *Arabidopsis* genome encodes more than 200 leucine-rich repeat (LRR) RLKs with an organization of functional domain organization similar to that of animal receptor kinases and more than 600 RLKs, which is the largest kinase superfamily in plants and a major part of the PSTKs (90). Thus, they do not possess the typical genes encoding components similar to those of the major animal signalling pathways, which interact with ligands at the plasma membrane and subsequently mediate the extensive cascade of downstream Tyr phosphorylations. Receptor PTKs (RTKs), epidermal growth factor receptor (EGFR), Hedgehog, Jak/STAT, Notch, Wingless, and also transmembrane receptor-like PTKs from RTK-Ras pathways are lacking in plants.

In contrast, signalling in *Arabidopsis* involves a huge number of protein serine/threonine receptor kinases, i.e. RLKs that were first discovered in the plant kingdom, and two-component histidine kinases present in both bacteria and plants. The Ser/Thr RLKs make up roughly one-third of all PSTKs. This composition of kinase specificity seems to be a characteristic feature of higher

plants and it is speculated that signalling mechanisms have diverged considerably in plants compared to other phyla due to the vast number of environmental and intracellular signals to be integrated by plants, which mirrors the same vague theory provided to explain extensive gene duplication and redundancy in plants (76).

The presence of a large number of PSTKs has lead to the opinion that plant Tyr phosphorylation might be less frequent than in animals (17, 95). However, bioinformatics helped to identify putative DSKs and also candidates for PTKs (96–98). This might hint towards a situation similar to that of *Saccharomyces cerevisiae*, which also lacks PTKs but harbours a battery of DSKs. On the contrary, only recently, mass spectrometry indicated that Tyr phosphorylation is as extensive in plants as it is in animals (17). Nevertheless, a significant difference is noted between animals and plants regarding the function of protein phosphorylation in signal transduction (19).

3. Biological Roles of Kinases

Almost every aspect of cellular life relies on kinase function. A paradigm is signalling networks that enable a cell to quickly respond to environmental stimuli and internal cues such as in nutrient and hormone reception and stress sensing. Besides being fast and reversible, protein phosphorylation is tightly regulated, highly specific, and allows signals to be sustained or attenuated via amplification, feedback, and crosstalk. Target protein phosphorylation often results in the modification of its activities that in turn might serve as another downstream signal for further changes in a cascade of protein activities. In this context, protein phosphorylation and dephosphorylation reactions play a pivotal role (99).

In addition to that, also protein stability is often regulated by the state of phosphorylation. Target proteins may contain phosphoacceptor sites that function as so-called "phosphodegron motifs." These in turn can be recognized by 14-3-3 proteins and/or SCF (S-phase kinase-associated protein (Skp)-Cullin-F-box containing complex), a E3 ubiquitin ligase of the ubiquitin proteasome system (UPS). This ubiquitin ligase catalyzes target protein ubiquitination, ultimately leading to its degradation by the proteasome, a way of rapid depletion (100). The COP9 signalosome (CSN) regulates, for example, the ubiquitin-dependent degradation of transcription factors via phosphorylation (101–103) and phosphodegron-containing cyclins (104, 105), and CDK-inhibitors (106) can be destructed in a similar way. On the other hand, phosphorylation events can also increase the stability of a protein such as for transcription factors which are substrates of c-Jun (102, 107).

The specificity in signalling is, in part, achieved by two means. Catalytic specificity of protein kinases and phosphatases provides the basis for site-specific phosphorylation and dephosphorylation, respectively (34). Specific tyrosine and serine/threonine phosphorylation sites and their surrounding sequences, in turn, provide selective binding sites for conserved protein modules found in most cytoplasmic signalling molecules (8, 33). Identifying the sites of protein phosphorylation and the nature of the phosphorylated residue is therefore crucial in the study of signal transduction.

Phosphorylation and dephosphorylation of a protein often serves as an "ON/OFF" switch in the regulation of cellular activities, and protein phosphorylation appears to be involved in almost all signalling pathways throughout the kingdoms. For optimal function within the phosphorylation network, kinases and phosphatases must strike a balance in any given cell. However, only a very small fraction of the thousands of protein kinases and phosphatases in plants have yet been studied experimentally, but critical functions for these enzymes have been demonstrated many times in growth and development.

3.1. Examples

This section and the kinases mentioned hereafter represent a couple of examples related to this book and are referenced with those chapters presenting technologies for their study.

3.1.1. CDKs

Similar to the highly expanded families of RLKs and the MAPK cascade members, the number of putative CDKs and CKL/CLK/LAMMERs is also increased in plants (82). *Arabidopsis* has one central CDK, namely CDKA;1 (108, 109). The main CDKs such as human Cdk1, yeast $Cdc2^{+}$/Cdc28p, and also *Arabidopsis* CDKA;1, contain the archetypically conserved PSTAIRE hallmark in the C-helix of the N-terminal cyclin-binding domain (residues 42–57 in human Cdk2; (110)). However, so-called non-PSTAIRE-Cdks with different functions also exist.

Harashima et al. present detailed procedures both on expression of *Arabidopsis* CDKA;1 in insect cells and tobacco cell suspension cultures in Chapter 4. Furthermore, they describe immunoprecipitation-coupled and affinity purification-based kinase assays on purified CDK/Cyclin complexes. In Chapter 14, Pusch et al. give a detailed view of BiFC assays on CDKA;1 phospho-site mutants, and Dissmeyer and Schnittger highlight a complete procedure on phospho-site substitution, mutant transformation and in vitro kinase assays of *Arabidopsis* CDKA;1 in Chapter 6 of this book.

3.1.2. MAPK

MAPK cascades are highly conserved modules in all eukaryotes. In plants, these participate in hormonal responses (auxin, abscisic acid (ABA), and possibly ethylene and cytokinins), cell cycle regulation, pathogen defence (by pathogens themselves or pathogen-derived elicitors), wounding response, hypersensitive

response (HR)-like programmed cell death (PCD), and mediate external stresses (111, 112). In addition, MAPK cascades are also involved in responses to a diversity of physical environmental stimuli and abiotic stresses including cold, heat, high UV, salinity, drought, and reactive oxygen species (ROS) (reviewed in refs. 113, 114) and function in interplay with CDPKs (reviewed in ref. 115).

Arabidopsis MAPK 6 (MPK6) can regulate signalling of the gaseous phytohormone ethylene via phosphorylation of an in vivo substrate transcription factor (116) and control ethylene synthesis via phosphorylation of ethylene biosynthetic enzymes (117, 118). Chapters 3 and 5 by Sonkoly et al. and Dóczi et al. focus on the purification of *Arabidopsis* MAP kinase 9 (MPK9) and on general MAPK activity and reporter gene assays. In Chapter 9, Li et al. describe MAPK inhibitor studies to investigate their role in PCD in pollen, and Salomon et al. write about chemical genetics approaches for the study of tomato MPK3 in Chapter 10. Umbrasaite et al. give a detailed protocol for inhibition of *Arabidopsis* PP2C-type protein phosphatase which counteracts MPK6 activity in order to regulate developmental and stress signalling pathways in Chapter 8. In addition, in Chapter 15, Böhmer et al. present the generation of *Arabidopsis* plants overexpressing inhibitor-sensitive CALCIUM-DEPENDENT PROTEIN KINASE 1 (CPK1).

3.1.3. RLKs

Genetic and biochemical studies indicate specific functions for plant PSTKs in development and in sensing of environmental stimuli. The LRR-RLKs ERECTA, CLAVATA 1 (CLV1), TOUSLED, and HAESA are required to establish important developmental processes such as apical meristem identity and organ morphology (119–123). Proteomic approaches complemented genetical studies and this interplay lead to a breakthrough in the identification of members of the signal transduction cascade of growth-promoting brassinosteroid (BR) hormones in plants. This includes BR perception by the putative receptor of BR, BRASSINOSTEROID INSENSITIVE 1 (BRI1) receptor kinase at the cell surface (reviewed in refs. 4, 76, 124). Chapters 11 (Hink et al.), 12 (Geldner et al.), and 13 (Kwaaitaal et al.) describe spectroscopical and microscopical techniques used to study some of these RLKs, e.g. by addressing receptor kinase dimerization kinetics, stability, and localization.

Genetic evidence has identified the *Brassica napus* (rape seed) S-receptor-like kinase (SRK) as the female determinant of the self-incompatibility (SI) recognition response (reviewed in refs. 125–127). The SI recognition response in pollen–pistil interactions in the pollination process of plant sexual reproduction is based on secreted S-locus glycoprotein (SLG) and a transmembrane SRK (reviewed in refs. 123, 126). Inhibitor studies on the SI response are described in Chapter 9 by Li et al.

3.1.4. His Kinases

Ethylene is perceived through a two-component His kinase receptor (128) which interacts physically with CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) kinase, a Raf MAP3K/MEKK, the upstream element of a MAPK module. The three *Arabidopsis* cytokinin receptors ARABIDOPSIS HISTIDINE KINASE 2 and 3 (AHK2 and 3) as well as CYTOKININ RESPONSE 1 (CRE1; (129)) are His kinases like the five ethylene receptors ETHYLENE RESPONSE 1 and 2 (ETR1/2), ETHYLENE SENSOR 1 and 2 (ERS1/2), and ETHYLENE INSENSITIVE 4 (EIN4; (130–132)). These kinases are altogether vital for proper plant development. The *Arabidopsis* histidine kinase CYTOKININ-INSENSITIVE 1 (CKI1) was recently shown to be required for female gametophyte and vegetative growth (133), and a bacterial assay to study plant sensor histidine kinases is presented in Chapter 7 by Spíchal et al.

4. Databases and Webtools

See Table 6

5. Note

1. According to Champion et al., a new nomenclature for the plant MAPK cascade was suggested (81): MAPK is now MPK, MEK is MAPKK/MAP2K, and MEKK is MAPKKK/MAP3K. Moreover, for *Arabidopsis*, MKK for MEK/MAP2K and MPK for MAPK were introduced.

Acknowledgments

We thank Karsten Niefind, Emily Mc Callum, and Anna Richter for critical reading and helpful comments on the manuscript. This work was supported by an Action Thématique et Incitative sur Programme (ATIP) grant from the Centre National de la Recherche Scientifique to A.S.

Table 6
List of useful online databases and tools

Name	Description	Availability	References
Plant sequence databases			
BrachyBase	Displays the JGI 8× *Brachypodium distachyon* genome assemblies with MIPS/JGI gene predictions, *Brachypodium* EST alignments, Illumina-based RNAseq data, and empirical TAU gene model predictions	http://www.brachybase.org/	
BRACHYPODIUM.ORG	The *Brachypodium distachyon* Information Resource. Genetic stocks, tools, search, brachy sequence resource	http://www.brachypodium.org/	
Brachypodium genome database	Genome view, comparative map viewer, data overview, search and search for protein domains	http://mips.helmholtzmuenchen.de/plant/brachypodium/index.jsp	
Lotus japonicus genome database	Genome view, comparative map viewer, data overview, search, and search for protein domains	http://mips.helmholtzmuenchen.de/plant/lotus/index.jsp	
MAtD	The MIPS *Arabidopsis thaliana* genome database. Genome view, comparative map viewer, data overview, search and search for protein domains, and chromosomal heatmaps	http://mips.helmholtzmuenchen.de/plant/athal/index.jsp	
Medicago MT3 genome database	UMN Medicago resources. Information about the genome sequencing project (MT3.0 assembly release) Medicago and other legume resources and links	http://www.medicago.org	
MaizeGDB	Maize genetics and genomics database	http://www.maizegdb.org/	
MaizeSequence	Maize genome browser	http://maizesequence.org/	
MGSP	The maize genome database. Genome view, comparative map viewer, data overview, search and search for protein domains	http://mips.helmholtz-muenchen.de/plant/maize/index.jsp	(147)
modelcrop.org	The *Brachypodium distachyon* comparative genomics resource. Comparative genomics tools and resources	http://www.modelcrop.org/	

(continued)

Table 6
(continued)

Name	Description	Availability	References
MOsDB	The rice genome database. Genome view, comparative map viewer, data overview, search and search for protein domains, and comparative map viewer	http://mips.helmholtzmuenchen.de/plant/rice/index.jsp	
PlantsDB	MIPS plant database for monocots (maize, rice, *Sorghum*, *Brachypodium*) and dicots (*Arabidopsis*, *Medicago*, *Lotus*, tomato)	http://mips.helmholtzmuenchen.de/plant/	
PoplarDB	Poplar functional genomics database with search, BLAST, and DB browser	http://mycor.nancy.inra.fr/poplardb/	
PopulusDB	Resource for tree genomics, including Populus DNA microarray database	http://www.populus.db.umu.se/	(86)
Sorghum genome database	Genome view, comparative map viewer, data overview, search and search for protein domains, and comparative map viewer	http://mips.helmholtzmuenchen.de/plant/sorghum/index.jsp	
TAIR	The *Arabidopsis* Information Resource. The world's leading database of genetic and molecular biology data for the model higher plant *Arabidopsis thaliana* and important resources. Complete genome sequence, gene structure, gene product information, metabolism, gene expression, DNA and seed stocks of mutants, genome maps, genetic and physical markers, publications, and information about the *Arabidopsis* research community	http://www.Arabidopsis.org/	
Tomato genome database	Genome view, comparative map viewer, data overview, search and search for protein domains, and comparative map viewer	http://mips.helmholtzmuenchen.de/plant/tomato/index.jsp	
UrMeLDB	The Medicago MT3 genome database. Genome view, comparative map viewer, data overview, search and search for protein domains	http://mips.helmholtzmuenchen.de/plant/medi3/index.jsp	
UK CropNet Databases	Combination of six UK CropNet databases as CropSeqDB with data on crop plants and grasses (*Arabidopsis* Genome Resource, BarleyDB, BrassicaDB, FoggDB, MilletGenes)	http://ukcrop.net/db.html	

(continued)

Table 6 (continued)

Name	Description	Availability	References
Plant phosphorylation			
PepBase	A peptide database with MS/MS spectra, consists of experimental data focused on phosphoproteomics and membrane proteomics. PepBase is divided into two tools: Phospho-PepBase and MembranePepbase	http://pepbase.iab.keio.ac.jp/phospho/msb/	
PhosPhAt 3.0		http://phosphat.mpimp-golm.mpg.de/	(150, 151)
PlantsP	Functional genomics database for plant phosphorylation, comprises information from all plant species (i.e. kingdom *viridiplantae*). PlantsP provides a resource for information on a collection of T-DNA insertion mutants (knockouts) in each kinase and phosphatase, primarily in *Arabidopsis thaliana*	http://plantsp.sdsc.edu/	(74, 75)
Plant tools			
ABRC	*Arabidopsis* Biological Research Centre. Resource centre at the Ohio State University which collects, reproduces, preserves, and distributes seed and DNA resources of *Arabidopsis thaliana* and related species. Stock information and ordering for the ABRC are fully integrated into TAIR	http://Arabidopsis.org	
Arabidopsis eFP Browser	"Electronic Fluorescent Pictograph" browser for exploring and analyzing large-scale biological data sets	http://www.bar.utoronto.ca/efp/	(152)
Genevestigator	Database of gene expression and regulation in a broad variety of contexts summarizing and interpreting microarray data	https://www.genevestigator.com/	
KOME	Knowledge-based *Oryza* molecular biological encyclopaedia full-length cDNA database; full-length cDNA clones from rice (*Oryza sativa* L. ssp. japonica cv. Nipponbare)	http://cdna01.dna.affrc.go.jp/cDNA/	(153)
NASC	Nottingham *Arabidopsis* Stock Centre	http://Arabidopsis.info	
SIGNAL	Genomic DNA flanking sequences of the T-DNA insertion for most of these populations are searchable here	http://signal.salk.edu/cgi-bin/tdnaexpress	(154)

(continued)

Table 6 (continued)

Name	Description	Availability	References
TIGR	The Institute of Genomic Research	ftp://ftp.tigr.org/pub/data/Eukaryotic_Projects/o_sativa/	
TILLING	Targeting Induced Local Lesions in Genomes; *Arabidopsis* TILLING Project (ATP)/Seattle TILLING Project (STP)	http://tilling.fhcrc.org/	(155–157)
Kinase databases and tools			
kinase.com	Genomics, evolution, and function of protein kinases	http://kinase.com/	
KinBase	The kinase database at Sugen/Salk	http://kinase.com/kinbase/	(67)
KinG	Database of protein kinases in genomes, kinase domains identified. Comprehensive collection of serine/threonine/tyrosine-specific kinases and their homologues identified in various completed genomes using sequence and profile search methods. Catalytic domain search tool	http://hodgkin.mbu.iisc.ernet.in/king/	(158)
Kinomer	Characterized Kinomes. Protein kinase classifcation HMM library and database. Multilevel HMM library that models these protein kinase groups. It allows accurate identification of protein kinases and classification to the appropriate kinase group	http://www.compbio.dundee.ac.uk/kinomer/kinomes.html	(98, 159)
Kinomer Search Tool	Precalculated Kinome Search	http://www.compbio.dundee.ac.uk/kinomer/bin/kinomes.pl	(98, 159)
KSD	Kinase Sequence Database. Collection of protein kinase sequences grouped into families by homology of their catalytic domains	http://sequoia.ucsf.edu/ksd/	
MOLBINFO	Molecular Biology and Bioinformatics Unit	http://www.bio.unipd.it/molbinfo/PTKtable.html	
PhosphoBase	See Phospho.ELM		
Phospho.ELM	Formerly PhosphoBase; a database of phosphorylation sites includes the Phospho.ELM BLAST Search tool	http://phospho.elm.eu.org/	(160)

(continued)

Table 6 (continued)

Name	Description	Availability	References
Protein Kinase Resource	Collection of services, tools, and information about kinases	http://pkr.sdsc.edu/html/index.shtml	(161)
RKD	Rice Kinase Database. Platform to display user selected functional genomic data on a phylogenetic tree	http://rkd.ucdavis.edu	(78)
Protein and proteomics			
Brenda	Enzyme Information System	http://www.brendaenzymes.org/	
ConSurf	Server for the Identification of Functional Regions in Proteins by Surface-Mapping of Phylogenetic Information	http://consurf.tau.ac.il/	
EBI	Analysis Tools at European Bioinformatics Institute	http://www.ebi.ac.uk/Tools/index.html	
ExPASy	Proteomic tools	http://www.expasy.ch/tools/	
Search tools			
BLAST	Basic Local Alignment Search Tool of the National Center for Biotechnology Information; finds regions of similarity between biological sequences	http://www.ncbi.nlm.nih.gov/	
BLAST2	Advanced BLAST2 Search Server	http://dove.embl-heidelberg.de/Blast2/	
BLASTP	Protein BLAST to search protein databases using a protein query	http://www.ncbi.nlm.nih.gov/	
PatScan	Pattern scan of proteins or nucleotides	http://www-unix.mcs.anl.gov/compbio/PatScan/HTML/patscan.html	
Peptide Search	Protein identification by peptide mapping or peptide sequencing	http://www.mann.embl-heidelberg.de/GroupPages/PageLink/peptidesearchpage.html	
PROPSEARCH	Compositional search using a sequence	http://www.infobiosud.univ-montp1.fr/SERVEUR/PROPSEARCH/propsearch.html	

(continued)

Table 6
(continued)

Name	Description	Availability	References
PSI-BLAST	Position Specific Iterative BLAST, identification of protein kinases		(162)
RPS-BLAST	Reverse position specific BLAST; identification of protein kinases		(162)
Databases of structural protein properties			
CDD	Conserved Domain Database: a curated Entrez database of conserved domain alignments		(163)
Consensus sites	ScanProsite	http://www.expasy.org/tools/scanprosite/	
DOMO	Database of homologous protein domains	http://www.infobiogen.fr/srs6bin/cgi-bin/wgetz?-page+LibInfo+-lib+DOMO+-newId	
PDB	The Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank. Obtain PDB structure	http://www.pdb.org/	
PFAM	Protein families database of alignments and HMMs (hidden Markov models)	http://bioinformatics.weizmann.ac.il/Pfam/	(164)
PRODOM	The protein domain database	http://www.toulouse.inra.fr/prodom.html	
PROSITE	Database of protein families and domains	http://www.expasy.ch/prosite/	
SCOP	Structural classification of proteins	http://scop.mrc-lmb.cam.ac.uk/scop/	
Proteomics databases			
Matrix Science Mascot	(Searches based on mass spectrometry data, generally very fast!)	http://www.matrixscience.com/search_form_select.html	
MOWSE	At the HGMP-RC, searches based on peptide masses	http://srs.hgmp.mrc.ac.uk/cgi-bin/mowse	
Peptide Mass	Calculates mass from a sequence	http://us.expasy.org/tools/peptide-mass.html	

(continued)

Table 6 (continued)

Name	Description	Availability	References
ProFound	Searches based on mass spectrometry data	http://129.85.19.192/profound_bin/WebProFound.exe	
ProteinProspector	Searches based on mass proteometry data	http://prospector.ucsf.edu/	
SWISS-2DPAGE	Human 2-D PAGE databases for proteome analysis in health and disease	http://www.expasy.ch/ch2d/	
General sequence databases			
Animal Genome Size Database	Collection of services, tools, and information	http://www.genomesize.com/	
dictyBase	Central resource for Dictyostelid genomics	http://dictybase.org/	
DOGS	Database of Genome Sizes	http://www.cbs.dtu.dk/databases/DOGS/	
Uniprot	Universal Protein Resource	http://www.uniprot.org/	(165)
Interpro	Integrated database of predictive protein "signatures" used for the classification and automatic annotation of proteins and genomes, i.e. IPR011009 Protein kinase-like, IPR000719 Protein kinase, IPR001245 Tyrosine protein kinase, and IPR002290 Serine/threonine protein kinase	http://www.ebi.ac.uk/interpro/	
EBI	European Bioinformatics Institute	http://www.ebi.ac.uk/	
EMBL	European Molecular Biology Laboratory, service and tools Web pages	http://www.emblheidelberg.de/Services/index.html	
KEGG	Kyoto Encyclopaedia of Genomes and Genes	http://www.genome.jp	
Kimball's Biology Pages	Collection of services, tools, and information	http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/	
MIPS	Munich Information Center for Protein Sequences hosted by The Institute of Bioinformatics and Systems Biology (IBIS) of the Helmholtz Zentrum München	http://mips.helmholtzmuenchen.de/	

(continued)

Table 6 (continued)

Name	Description	Availability	References
NCBI	National Center for Biotechnology Information	http://www.ncbi.nlm.nih.gov/	
Royal Botanic Gardens	Kew Plant DNA C-values database	http://data.kew.org/cvalues/	
SWISS-PROT	Collection of services, tools, and information	http://www.expasy.org/sprot/	

Appendix A

Abbreviations and Synonyms

ABA	Abscissic acid
AGC	Group consisting of the cyclic nucleotide-dependent family (PKA, PKG), the PKC family and the ribosomal S6 kinase family
AHK2/3	ARABIDOPSIS HISTIDINE KINASE 2/3
APC/C	Anaphase-promoting complex/cyclosome
APK	Atypical PK
βARK	G-protein-coupled β-adrenergic RK
ATM	*Ataxia telangiectasia* mutated kinase
ATR	*Ataxia telangiectasia* and Rad3-related kinase
BiFC	Bimolecular fluorescence complementation
BR	Brassinosteroid
BRI1	BRASSINOSTEROID INSENSITIVE 1 receptor kinase
Cak	Cdk-activating kinase
CAKAK	Cak-activating kinase
CaM	Calmodulin
CaMII	Ca^{2+}/CaM-dependent kinase II
CaMK	Ca^{2+}/CaM-regulated kinase
CaMK	Group consisting of CaMK interacting proteins and SNF1 proteins
CaMKK	CAMK kinase
cAMP	Cyclic adenosine monophosphate
cAPK	cAMP-dependent PK
Cdc	Cell division control
Cdk	Cyclin-dependent (protein) kinase
CDPK	Ca^{2+}-dependent PK/CaM-like domain PK
cGMP	Cyclic guanosine monophosphate
CK1/2	Protein kinase 1/2, name derived from Casein Kinase 1/2

CKI1	CYTOKININ-INSENSITIVE 1
CKL/CLK	Cdk-like kinases
CLV1	CLAVATA 1
CPK1	*Arabidopsis* CALCIUM-DEPENDENT PROTEIN KINASE 1
CRE1	CYTOKININ RESPONSE 1
CTR1	CONSTITUTIVE TRIPLE RESPONSE 1
DNA-PK	DNA-dependent protein kinase
DSK	Dual-specificity kinase
DYRK	Dual-specificity Tyr-regulated kinase
EEF2K	Elongation factor-2 (eEF-**2**) kinase
EF	Elongation factor
EGFR	Epidermal growth factor receptor
EIN4	ETHYLENE INSENSITIVE 4
EPK	Eukaryotic PK
ERK	Extracellular signal-regulated kinase (MAPK)
ERS1/2	ETHYLENE SENSOR 1/2
ETR1/2	ETHYLENE RESPONSE 1/2
GSK	Glycogen synthase kinase
IRAK	Interleukin-1 receptor associated kinase
JAK/STAT	Janus family of TK/signal transducer and activator of transcription
LAMMER	Subfamily of kinases possessing a LAMMER (or related) amino acid sequence
LCK	Lymphocyte-specific PTK
LRR	Leucine-rich repeat
MAP2K	MAPK kinase, MAP kinase kinase, MAPKK, now MEK in plants and MKK in *Arabidopsis**
MAP3K	MAP2K kinase, MAP kinase kinase kinase, MAPKKK, also known as MEKK*
MAP4K	MAP3K kinase, MAP kinase kinase kinase kinase, MAPKKKK*
MAPK	Mitogen-activated PK; now MPK in plants*
MAPKK	MAPK kinase*
MAPKKK	MAPKK kinase*
MEK	MAP/ERK kinase, MAPKK, also known as MKK/MAP2K*
MEKK	MEK kinase, MAPKKK, canonical MAP3K*
MHCK	Myosin heavy chain kinase
Mik1	*Schizosaccharomyces pombe* mitotic inhibitor kinase 1
MKK	New name for *Arabidopsis* MEK/MAP2K/MAPKK*
MKK	New name for *Arabidopsis* MAP2K*
MLK	Mixed lineage kinase
MLK	Mixed lineage kinase
MPK	New name for *Arabidopsis* MAPK*
mTOR	Molecular target of rapamycin

Myt1	Membrane-associated Tyr/Thr PK 1
NEK	NIMA/NIM-A related kinase (NRK)
NIMA	"Never in mitosis A" (NIM-A)
PDGFR	Platelet-derived growth factor receptor
PDHK	Pyruvate dehydrogenase (acetyl-transferring) kinase
PEPRK	PPCK-related kinases
PhK	Phosphorylase kinase
PIKK	Phosphatidyl inositol 3′ kinase-related kinase
PK	Protein kinase
PKA, C, G	Cyclic nucleotide-regulated protein kinase A, C, or G
PKA/B/C	Protein kinase A/B/C
PKACα	PKA catalytic subunit α
PKAK1	Pyruvate dehydrogenase kinase
PKL	Protein kinase-like
PPCK	Phosphoenolpyruvate carboxylase kinase
PPK1	Phosphatidylinositol-4-phosphate 5′ kinase
Prk	Phosphoribulokinase
PRKIN	Protein kinase also called MHK
PSTK	Protein serine/threonine kinase
Pti1	Pto-interacting 1
PTK	Protein–tyrosine kinases
Raf	"Rapidly growing fibrosarcoma or rat fibrosarcoma," cellular homolog of oncogene products from murine sarcoma virus, or cellular homolog of v-raf, the transforming gene from an avian retrovirus (MAP3K)
Rb	Retinoblastoma protein
RD	Kinase kinase containing invariant Asp residue as catalytic base preceded by an Arg
RGC	Receptor guanylate cyclase
RIO	Right open reading frame
RK	Receptor kinase
RLK	Receptor-like kinase (protein Ser/Thr RLK)
ROS	Reactive oxygen species
RTK	Receptor Tyr kinase
SH	Hydrosulphide or sulfhydryl group (in Cys)
SHAGGY	GSK from *Drosophila*
SI	Self-incompatibility
SILAC	Stable isotope labelling
SLG	S-locus glycoprotein
SMG-1	Suppressor of morphogenesis in genitalia-1
SNF1	Sucrose non-fermenting 1
SnRK	SNF1-related kinase
SRK	S-receptor-like kinase
STE	Family including MAP2K, MAP4K, CDCs, MEKK, and related sequences; kinase group consisting of Sterile 20 and p21Ras-activated PK (MAP4K)
TGFβ	Transforming growth factor-β

TGFβR1	Transforming growth factor-β receptor kinase 1 (TGFBR1)
TK	Tyr kinase
TKL	Tyr kinase-like
TRPM	Transient receptor potential ion channel melastatin
VEGFR	Vascular endothelial growth factor receptor
WAK	Cell wall-associated kinase

*New MAPK cascade nomenclature after (81)

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Plant Kinases
Methods and Protocols
Dissmeyer, N.; Schnittger, A. (Eds.)
2011, X, 285 p. 39 illus., Hardcover
ISBN: 978-1-61779-263-2
A product of Humana Press