of the kinase, and disruption of this pseudo-substrate domain is an important component in kinase activation [26]. This mechanism is not confined to protein kinases [27]. The identification of a pseudo-substrate domain in the oncogene MDM2, whose affinity for MDM2 can be modified by phosphorylation, expands on the class of proteins that can be regulated by intrasteric mechanisms and has interesting implications for control of other MDM2-binding proteins like p53, p300, E2F, p73 and Rb.

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Protein Sequence Motif

The KIND module: a putative signalling domain evolved from the C lobe of the protein kinase fold

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A novel putative interaction domain – KIND (kinase non-catalytic C-lobe domain) – has been identified as being similar to the C-terminal protein kinase catalytic fold (C lobe). Its presence at the N terminus of signalling proteins and the absence of the active-site residues in the catalytic and activation loops suggest that it folds independently and is likely to be non-catalytic. The occurrence of the novel domain only in metazoa implies that it has evolved from the catalytic protein kinase domain into an interaction domain possibly by keeping the substrate-binding features.

The *Drosophila melanogaster* protein p150-Spir (CAB62901), which is phosphorylated by the c-Jun

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N-terminal kinase (JNK) kinase [1], has been described as an important regulator of the actin cytoskeleton [1,2] and a mediator of vesicular transport [3]. The Spir proteins form a well-conserved family in animals. In the central region they contain a WH2 (Wiskott–Aldrich syndrome homology region 2) domain cluster, which is known to bind actin [4,5]. The C-terminal region encodes a modified FYVE zinc-finger domain, which directs the subcellular localization of the proteins [3] (Fig. 1a).

Sequence analysis of the Spir protein N-terminal region

To elucidate the features of the Spir N-terminal sequence, a search of the non-redundant protein database with PSI–BLAST [6] using the first 350 residues of *Dm* p150-Spir was performed. The putative orthologues in *Anopheles gambiae* (EAA12347, $E = 10^{-23}$), *Homo sapiens* (Spir-1, BAA86449, $E = 10^{-13}$; Spir-2, CAD19439, $E = 10^{-7}$), *Rattus norvegicus* (Spir-1, XP_225864, $E = 10^{-13}$; Spir-2, XP_226553, $E = 10^{-6}$) and *Mus musculus* (Spir-1, XP_140409, $E = 10^{-11}$; Spir-2, CAD30509, $E = 10^{-7}$) genomes were first retrieved. After detecting the known homologue PEM-5 (BAA36293, $E = 10^{-5}$), one of the maternal factors required for pattern formation and cell specification in the embryo of *Ciona savignyi* [7], the search converged. In the twilight zone just below the threshold, a match of ~200 residues with two regions of the uncharacterized human protein KIAA1768 (BAB21859, $E = 0.07$) prompted a more detailed analysis. To test the significance of this similarity, we did a reciprocal search of the non-redundant database using the best matching region of the KIAA1768 protein (residues 30–220). After retrieving members of a protein phosphatase family in several animals (for *Hs*, BAA04752, $E = 10^{-4}$), the Spir orthologues in different species with statistical significance (for *Hs* Spir-1 and Spir-2 $E = 10^{-5}$ and 10^{-4}, respectively, both found in the first iteration) and PEM-5 ($E = 10^{-5}$ in the second iteration), the region significantly matched various catalytic domains of protein kinases. The best similarity was found to p21-activated kinase (PAK; $E = 10^{-4}$ in the second iteration), a serine threonine phosphotransferase.

Evolutionary relationship between the new domain and the catalytic region of the kinase fold

The protein kinases, which all transfer the γ-phosphate from ATP to a receiving hydroxyl group of the target protein but differ in the activation and/or inactivation processes, represent one of the most abundant families in the eukaryotic genomes [8]. Multiple sequence alignments of some representatives of the different protein kinase subfamilies identified 11 major conserved motifs [9,10]. The first 3D structure solved, that of the Ser/Thr protein kinase A (PKA) [11], showed those motifs as sites important for the catalytic function, substrate recognition, nucleotide binding, and structural and folding properties (Fig. 2). The fold harbours two structurally independent subdomains: a short N lobe formed by a β sheet and an α helix that contributes to the binding of ATP, and a larger and mainly helical C lobe that contains the catalytic residues and the activation loop. The two lobes are joined by a short linker region (Fig. 1b). The matches between the Spir protein and the kinase domain cover the entire C lobe, but do not extend to the N lobe and the linker region (Fig. 2). Both the consensus sequence and the secondary-structure prediction, calculated using the multiple alignment of all the other sequences except the kinases [12], agree well with those determined for protein kinases (Fig. 2). The only exceptions are the motifs implicated in catalysis (i.e. the catalytic loop, the activation loop, and subdomains VIb and VII). The significant similarity and the structural context clearly imply a common ancestor between the region analyzed and the C-lobe subdomain of the protein kinases. The absence of the N lobe and the essential catalytic residues (the RD and DFG motifs; Fig. 2) suggests that the domain does not have any kinase activity. Moreover, as no invariant residue is dectectable from the alignment, another catalytic function is unlikely. Consequently, we named the new region the KIND domain, for kinase non-catalytic C-lobe domain. Other instances of a structural fold shared between enzymatic and non-enzymatic proteins have been already described [13–17]. In the majority of the cases reported so far, the non-enzymatic domain probably arose from a pre-existing

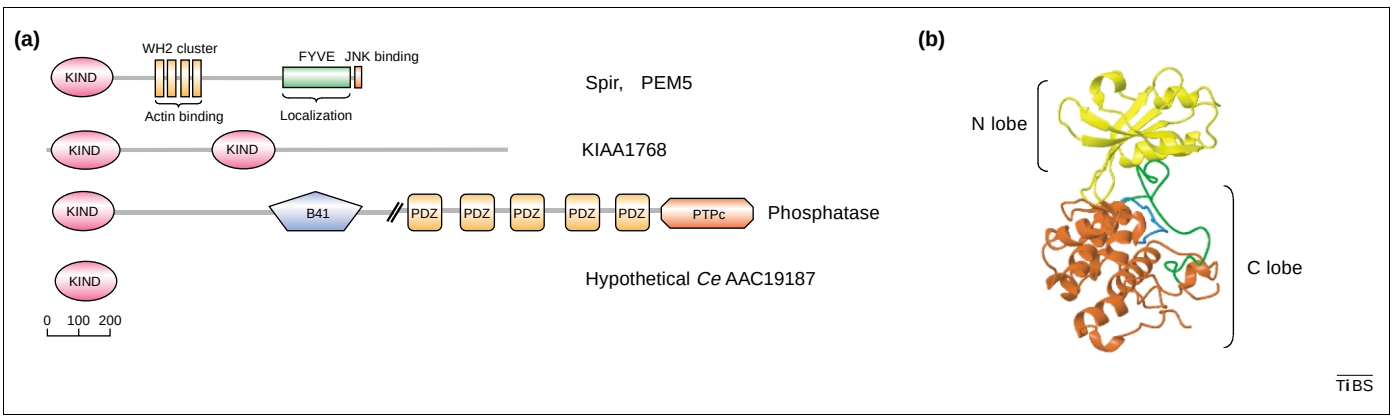

Fig. 1. (a) Representation of the different domain architectures containing one or more KIND (kinase non-catalytic C-lobe domain) modules. The domains are named according to the SMART database (http://smart.embl-heidelberg.de) [24,25]. Note that, in the proximity of the *Caenorhabditis elegans* chromosome IV region coding for the hypothetical protein AAC19187, a putative phosphatase gene has been predicted (AAF60494). Nevertheless, a genewise analysis using the human phosphatase BAA04750 was unable to detect a unique orthologue with the complete domain composition. Abbreviations: B41, band 4.1 homologue domain; JNK, c-Jun N-terminal kinase; PDZ, domain present in PSD-95, Dil and ZO-1/2; PTPc, protein tyrosine phosphatase catalytic domain; WH2, Wiskott–Aldrich syndrome homology region 2. (b) The 3D structure of p21-activated kinase 1 (PAK1) [20] is reported as an example of the kinase structural fold. The activation and the catalytic loops in the C lobe are labelled in green and blue, respectively. The 3D representation was realized using the MolMol program [26].

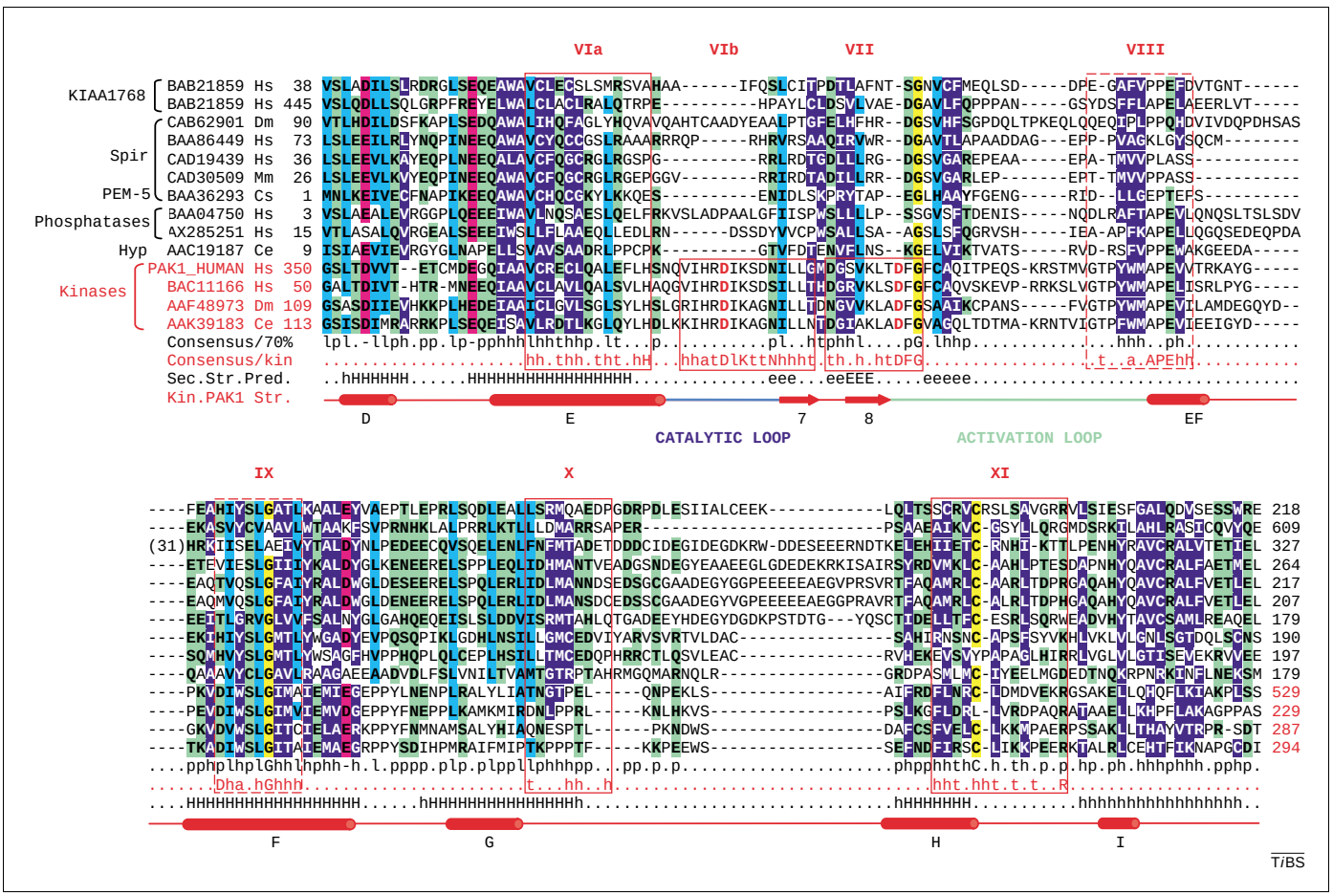

Fig. 2. Multiple sequence alignment of KIND (kinase non-catalytic C-lobe domain)-containing proteins with <80% of sequence identity. The alignment has been built taking non redundant representatives of each family retrieved by the PSI–BLAST analysis [6]. The sequences were aligned starting from the most similar ones and adding members of the other families. The final multiple alignment was manually refined. Sequences are indicated using the database accession number followed by the species. The starting and ending residues are reported before and after the corresponding sequence. The consensus in 70% of the sequences, not including the kinases, is reported below the alignment; h, l, p, t and – indicate hydrophobic, aliphatic, polar, turn-like and negative residues, respectively. Capital letters denote conserved amino acids. Hydrophobic residues are highlighted in blue, aliphatic residues in cyan, polar residues in green, negative residues in pink and other conserved residues in yellow. The two asparic residues in the protein kinase sequences involved in the catalytic function are in bold red type. The red boxes indicate the C-lobe subdomains (VIa–XI); following the classification reported by Hanks *et al.* [9], the conserved and variable motifs are indicated with unbroken and broken boxes, respectively. The protein kinase C-lobe consensus is in red text. The secondary-structure prediction (Sec.Str.Pred.) is taken from the consensus of the alignment, not including the kinases [12] (upper case indicates elements predicted with expected average accuracy >82%; lower case text indicates elements predicted with expected average accuracy <82%). The secondary-structure elements of the p21-activated kinase 1 (PAK1) 3D structure (PDB code: 1F3M) [20] are shown as red cylinders (α helices, αD–αI) and red arrows (β strands, β7–β8); the catalytic loop is indicated with a blue line and the activation loop with a green line. This multiple sequence alignment (alignment number ALIGN_000545) has been deposited with the European Bioinformatics Institute (ftp://ftp.ebi.ac.uk/pub/databases/embl/align/ALIGN_000545). Abbreviations: Ce, *Caenorhabditis elegans*; Cs, *Ciona savignyi*; Dm, *Drosophila melanogaster*;; Hs, *Homo sapiens*; Mm, *Mus musculus*; E, strands; H, helices. (See supplementary material; http://www.bork.embl.de/~ciccarel/KIND_aln.html).

enzymatic domain by mutation of catalytic residues [18]. This also seems to be the case for the KIND domain because of its presence only in animals and the ancient origin of the kinase domain, whose ancestor apparently predates the divergence of bacteria, archaea and eukaryotes [19]. After divergence from kinase, the KIND domain was apparently shaped not only by replacement of the catalytic residues, but also by deletion of the N lobe and the linker region. Thus, it represents another case in which the evolution of a putative interaction domain can be traced to a part of a catalytic one [18].

Functional implications

The precise functional role of the KIND domain remains to be elucidated, although interesting hypotheses can be formulated from the analysis of the protein families bearing it. The C-lobe domain mediates the interaction of kinases with activators, substrates and regulatory subunits [20,21], suggesting an interaction role for the KIND domain. It is known that the C-terminal region of p150-Spir protein interacts with the MAP kinase JNK [1]. The N-terminal KIND domain of the Spir proteins could mediate a back folding to the C-terminal region, which is responsible for the interaction with JNK, and thereby fix the protein in an auto-inhibited conformation as it has been shown for the functionally related Wiskott–Aldrich syndrome proteins (WASP) [22]. In addition, in phosphatases the KIND module could bind potential substrates. This hypothesis is supported by the fact that regions responsible for the regulation of their enzymatic activity have been localized outside the catalytic domain for several phosphatases (see [21,23] and references therein). Thus, uncovering the function of the KIND domain in the different proteins will reveal new insights into the mechanisms of signal transduction.

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The BON domain: a putative membrane-binding domain

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A novel conserved protein region – the BON (bacterial OsmY and nodulation) domain – is found in the bacterial osmotic-shock-resistance protein OsmY, a family of haemolysins, a group of nodulation specificity proteins and secretory channels, and several hypothetical proteins. Functional annotation in the literature suggests that it interacts with phospholipid membranes. A lack of catalytic residues in the sequence alignment supports the hypothesis that it is a binding domain.

Scanning genomes for novel domains is an effective method for elucidating both organism-specific information and more widespread biological processes [1]. We decided to carry out such a scan on *Deinococcus radiodurans* because it is renowned for its ability to withstand massive doses of radiation, and through similar mechanisms it can repair extensive DNA damage caused by rehydration from a desiccated state [2]. Our investigation identified a domain that is involved in osmotic-shock protection and other cell-membrane-localized processes through its interactions with phospholipid membranes.

Identification of the conserved region

A conserved repeat was identified in *D. radiodurans* protein DR0888 (SWISS-PROT accession number: Q9RVY3) using Prospero (http://www.well.ox.ac.uk/rmott/ARIADNE/prospero.shtml) and subsequent to

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