Histol Histopathol (2009) 24: 749-759

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Emerging biological functions of the Vaccinia-Related Kinase (VRK) family

Elke P.F. Klerkx[1], Pedro A. Lazo[2] and Peter Askjaer[1]

[1]Andalusian Centre for Developmental Biology (CABD), Spanish National Research Council (CSIC) – Pablo de Olavide University (UPO), Seville, Spain, and [2]Institute for Molecular and Cellular Cancer Biology, Cancer Research Centre, Spanish National Research Council (CSIC) – University of Salamanca, Campus Miguel de Unamuno, Salamanca, Spain

Summary. The Vaccinia-Related Kinases (VRKs) branched off early from the family of casein kinase (CK) I and compose a relatively uncharacterized family of the kinome. The VRKs were discovered due to their close sequence relation to the vaccinia virus B1R serine/threonine kinase. They were first described in phosphorylation of transcription factors that led to the discovery of an autoregulatory mechanism between VRK and the tumor suppressor transcription factor p53. The relevance of VRKs has broadened recently by introduction of its members as essential regulators in cell signaling, nuclear envelope dynamics, chromatin modifications, apoptosis and cellular stress response. Several phosphorylation substrates have been described, as well as the first positive and negative regulators of VRK. We provide an overview of the VRKs across species and discuss the wide diversity of cellular and organismal requirements for this kinase family.

Key words: Protein kinase, VRK, Cell signaling, Transcription factor, Nuclear envelope

Introduction

Protein phosphorylation of up to 30% of the proteome gives eukaryotic protein kinases control over many cellular processes. Phosphorylation of proteins generally results in their activation or inhibition, and may affect their localization and/or stability. The complement of protein kinases, also known as the kinome, accounts for up to 2% of protein encoding genes in eukaryotes and, due to its high conservation during evolution, provides an excellent super family for comparative studies across species (Manning et al., 2002). The human kinome consists of 518 protein kinases, of which 510 have a murine ortholog (Caenepeel et al., 2004). Protein kinases can be divided into 3 groups, based on their enzymatic properties. The majority of protein kinases transfer a phosphate group from ATP to the free hydroxyl group of the amino acids serine or threonine (ser/thr-specific protein kinases), whilst a smaller group acts on tyrosine (tyr-specific protein kinases), and a very small number of protein kinases acts on both (dual specificity kinases).

Several kinases act in sequential organization of signal transduction. Mitogen-activated protein kinase (MAPK) signaling represents the most well characterized system for signal transmission through ser/thr kinase complexes assembled on scaffold proteins that phosphorylate transcription factors (McKay and Morrison, 2007; Raman et al., 2007). Briefly, MAPK signaling is initiated by growth factor binding to receptor tyrosine kinases (RTKs) at the cell membrane (Sundaram, 2006; McKay and Morrison, 2007). This triggers RTK dimerization and autophosphorylation followed by sequential activation of downstream kinases such as Raf, Mek, and ERK. However, for most kinases, their substrates and interactions with signal transduction pathways are not known. In this context a striking example is represented by GSK3, identified almost forty years ago for its role in carbohydrate metabolism, and which is now recognized as an important player in ß-catenin regulation of epithelial to mesenchymal transition and cell invasiveness, among other pathways (Cohen and Frame, 2001).

Because of kinases' widespread impact on the activity of the proteome, tight regulation is indispensable for cellular and organismal homeostasis. Kinases are regulated by various mechanisms, ranging from the inhibition/activation at the transcriptional level to protein

Offprint requests to: Dr. Peter Askjaer, Centro Andaluz de Biología del Desarrollo, Consejo Superior de Investigaciones Científicas – Universidad Pablo de Olavide, Carretera de Utrera, km 1, Sevilla 41013, Spain. e-mail: pask@upo.es

modifications, such as phosphorylation, with many kinases possessing autophosphorylation ability. Kinases are counteracted by phosphatases, which enzymatically remove the phosphate group added by the kinase. Although a challenging research area due to the complexity and tight spatial and temporal regulation of kinases, the elucidating of their function is an important task for basic cellular and developmental biology, as well as for translational biomedical research. Probably one of the most intensively studied kinase regulators is the small GTPase Ras. The *Ras* gene was discovered about 30 years ago as a highly conserved gene, present in the genome from unicellular yeast to the multiple copies that can be found in humans (Karnoub and Weinberg, 2008). Ras is activated by the guanine nucleotide exchange factor Sos via RTK stimulation. Ras thereby recruits and activates Raf (McKay and Morrison, 2007). Numerous extracellular stimuli are transduced via RTK/Ras/MAPK signaling, which explains the crucial importance of this pathway in cell physiology. An estimated 20-30% of all human tumors are thought to contain a mutated Ras gene (Bos, 1989).

In this review we discuss the current state of research on vaccinia-related kinases (VRKs), more specifically, their function described so far in mammals, flies and worms. We discuss the biological impact of this important protein kinase family and possible future research prospects.

The family of vaccinia related kinases

The family of VRKs constitutes a new branch of the kinome, discovered by homology of its members to the vaccinia B1R serine/threonine kinase, which is important for viral replication (Traktman et al., 1989; Nezu et al., 1997). VRKs have branched off early from the family of casein kinase (CK) I (Manning et al., 2002). Three members are known to constitute the mammalian branch (VRK1, VRK2 and VRK3), whilst information from invertebrates is presented so far by a single ortholog in both fruit flies (nucleosomal-histone kinase-1; NHK-1), and in nematodes (VRK-1). There is no VRK ortholog known in yeast. Human VRK1 (hVRK1) consists of 396 amino acids and displays 40% sequence identity with the B1R kinase (Nezu et al., 1997). hVRK1 contains an N-terminal ser/thr protein kinase domain and a C-terminal nuclear localization signal (NLS) (Fig. 1A). Furthermore, its C-terminus contains an acidic region flanked by 2 basic regions (basic-acidic-basic (BAB) motif). EST database searches led to the discovery of a second ser/thr protein kinase named hVRK2 (Nezu et al., 1997). hVRK2 contains a transmembrane domain and two overlapping BAB motifs in its C-terminus (Fig. 1A). hVRK2 was recently found to have two differentially spliced isoforms, A and B, which have 508 and 397 amino acids, respectively, and differ in their C-terminus (Blanco et al., 2006). hVRK3, which was discovered by further database searches, consists of 474 amino acids (Nichols and Traktman, 2004) although skipping of hVRK3 exon 4 may produce a smaller protein of 424 amino acids. The hVRK3 and mouse mVRK3 enzymes are catalytically inert due to substitutions in several key residues within kinase motifs required for catalysis (Nichols and Traktman, 2004) (Fig. 1A). The murine VRK orthologs mVRK1-3, including two differentially spliced isoforms for mVRK1, were described by Zelko and colleagues (1998). The single *Drosophila melanogaster* VRK ortholog NHK-1 was found during biochemical purification of histone kinases (Aihara et al., 2004). The *nhk-1* gene encodes a 599 amino acid protein with an N-terminal kinase domain and the characteristic BAB motif

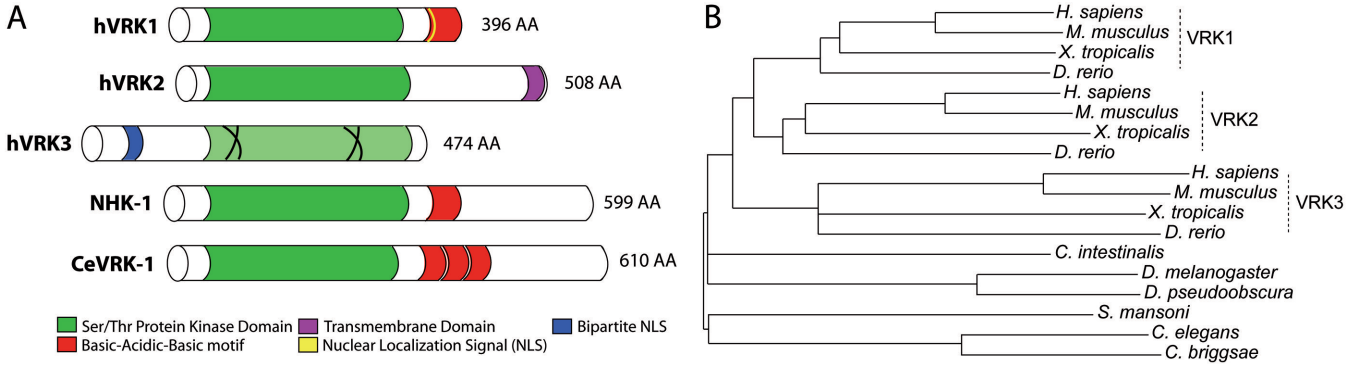

Fig. 1. The VRKs constitute a conserved protein family. **A.** All VRKs share a similar serine/threonine protein kinase domain (green) whilst the length of the extracatalytic domains varies. Human (h) VRK1 and hVRK3 contain a nuclear localization signal (yellow and blue) while hVRK2 has a transmembrane domain (purple). hVRK1, *D. melanogaster* NHK-1 and C. elegans VRK-1 contain one or multiple basic-acidic-basic (BAB) motifs (red). The kinase domain of hVRK3 contains several unusual kinase motifs (crosses) causing it to be catalytically inactive. **B.** Phylogenetic tree illustrating the similarity between VRKs of nematodes (*Caenorhabditis elegans* and *C. briggsae*), trematodes (*Schistosoma mansoni*), flies (*Drosophila melanogaster* and *D. pseudoobscura*), sea squirts (*Ciona intestinalis*), fish (*Danio rerio*), frogs (*Xenopus tropicalis*), mice (*Mus musculus*) and humans (*Homo sapiens*). Vertebrate genomes encode three VRK paralogs (VRK1-3) while invertebrates each have a single VRK gene. Modified from http://www.treefam.org/.

in its C terminus (Aihara et al., 2004) (Fig. 1a). *Caenorhabditis elegans* VRK (CeVRK-1) is a 610 amino acid protein, which has an N-terminal kinase domain and a C-terminal BAB motif (Gorjánácz et al., 2007). The completion of several genome-sequencing projects revealed that the increased number of VRK proteins in mammals presumably reflects the evolution of the VRK family. Thus, it appears that a single ancestral VRK gene was duplicated twice when the vertebrate branch evolved, leaving invertebrates with a single VRK gene and vertebrates with three VRKs (Fig. 1B).

Protein kinases are grouped into families based mainly on the structure of their catalytic domains. Kinase domains can be divided into 12 subdomains, I to XII, which contain patterns of conserved residues, also called motifs (Hanks and Hunter, 1995). Most protein kinases contain an APE motif within subdomain VIII, but in the CK family, from which the VRK family branched off, this is usually substituted by a SIN motif. In VRKs this motif has diverged even further to the loose consensus (P/S)XD (Nichols and Traktman, 2004). In both NHK-1 and CeVRK-1 a distinguishing tyrosine in subdomain VII (DYG) is substituted by a phenylalanine (DFG). The VRK proteins show high similarity between phylogenetic orthologs. hVRK1 for example shows an overall identity of 87% to mVRK1. hVRK2 and mVRK2 show 68% identity and hVRK3 and mVRK3 show 74% identity. The relatedness between paralogs is smaller with 44% identity between hVRK1 and hVRK2, 23% between hVRK2 and hVRK3 and 33% between hVRK1 and hVRK3 (Nichols and Traktman, 2004) (Fig. 1B).

hVRK1 and hVRK2 are ubiquitously expressed. Highest expression of hVRK1 is detected in tissues with high proliferation rates, such as the fetal liver, testis and thymus (Nezu et al., 1997). hVRK2 is highly expressed in fetal liver, skeletal muscles, pancreas, heart, peripheral blood leukocytes and testis (Nezu et al., 1997). mVRK1-3 are highly expressed in fetal liver and the hematopoietic system (blood, thymus and spleen). In adult murine tissues mVRK1-3 is highly expressed in liver, kidney and muscle tissues, whilst the hematopoietic system shows low expression levels (Vega et al., 2003). Relative expression levels of VRK proteins in most tissues seem to be correlated with high proliferation rates. However, the fact that VRK is also highly abundant in tissues with very low proliferation rates, such as the adult liver, implies a more complex regulation of VRK expression. At the subcellular level, human and murine VRK1 is found within the nucleus, but is also present in the cytosol in some cell lines (Nichols and Traktman, 2004; Vega et al., 2004; Valbuena et al., 2007a). VRK2A localizes to ER membranes and the nuclear envelope via its C-terminal transmembrane domain. Despite lacking a recognizable NLS, VRK2B is detected in both cytoplasm and nucleus (Nichols and Traktman, 2004; Blanco et al., 2006). VRK3 contains an N-terminal bipartite NLS and is localized exclusively to the nucleus (Nichols and Traktman, 2004). The dynamics of VRK localization during the cell cycle has so far only been addressed in flies and worms. In early S phase, *Drosophila* NHK-1 is mainly localized in the cytoplasm, but during prophase the protein changes its localization to condensing chromatin, where it stays during metaphase to be excluded from the chromatin at the end of mitosis (Aihara et al., 2004). CeVRK-1 also shows a highly dynamic pattern. During interphase, the protein is nuclear. However, shortly before the cell enters mitosis, the protein quickly relocates to the nuclear rim until nuclear envelope breakdown, when it binds to the chromatin and stays there until the end of mitosis (Gorjánácz et al., 2007) (Fig. 2A).

Regulation of transcription factors and tumorigenesis by VRKs

Posttranslational modifications, such as reversible phosphorylation, acetylation or methylation, play an important role in regulation of transcription factors. One of the most studied but complex transcription factors is p53. p53 is a tumor suppressor protein sometimes called the ‘gatekeeper of the genome’ due to its important function in maintaining cellular homeostasis by regulating cell cycle progression, apoptosis and DNA replication, in response to a wide variety of cellular stress signals (Bargonetti and Manfredi, 2002; Vousden and Lu, 2002). The levels and stability of p53 are mediated by negative regulators such as Mdm2 (hdm2 in humans) (Ashcroft and Vousden, 1999), COP1 and Pirh2 (Leng et al., 2003; Dornan et al., 2004) and also protein stabilization factors such as p300 (Yuan et al., 1999), MdmX (Stad et al., 2001) and HAUSP (Li et al., 2004). Interaction with Mdm2 targets p53 for degradation via the ubiquitin pathway. p53 is regulated by reversible phosphorylation in many serine or threonine residues in its N-terminal transactivation domain (Meek, 1999; Ryan et al., 2001; Toledo and Wahl, 2006). Interestingly, hVRK1 and hVRK2 phosphorylate p53 *in vitro* at its Thr18 residue, located in the transactivation domain (López-Borges and Lazo, 2000; Barcia et al., 2002; Vega et al., 2004; Blanco et al., 2006). This prevents the interaction of p53 with Mdm2 and instead promotes recruitment of p300 and thereby p53 stabilization (Vega et al., 2004). p53 and hVRK1 levels are inversely correlated in cultured cell lines; an increase in p53 levels results in the downregulation of hVRK1 by a mechanism that seems to be independent of Mdm2 and is likely not proteasome-mediated. Instead, an unknown gene targets hVRK1 to enter the endocytic lysosomal pathway (Valbuena et al., 2006). The autoregulatory loop between p53 and VRK1 is a basic cellular control mechanism under normal growth conditions. When cells are subject to stress, this balance will shift and result in p53 protein accumulation and trigger a p53 response (Vega et al., 2004). However, this autoregulatory loop is non-functional in a group of lung carcinomas with

inactivating p53 mutations (Santos et al., 2006; Valbuena et al., 2007b) and breast carcinomas {P.A.L., unpublished results}, resulting in high hVRK1 levels. In two types of cancer, head and neck squamous cell carcinomas (HNSCC) and in lung carcinomas, hVRK1 positively correlates with proliferation markers; survivin, cyclin A, Ki67 and topoisomerase in the case of HNSCC (Santos et al., 2006) and survivin and cyclin A in lung carcinomas (Valbuena et al., 2007b). In both cancer types, VRK1 is inversely correlated with p16. These case studies of the involvement of VRK1 in different types of cancers suggest that the role of VRK1 in transcription factor regulation and cell proliferation is complex and has multiple molecular faces. p53 transcriptional activity is regulated by transcriptional co-activators with acetylation function (Roy and

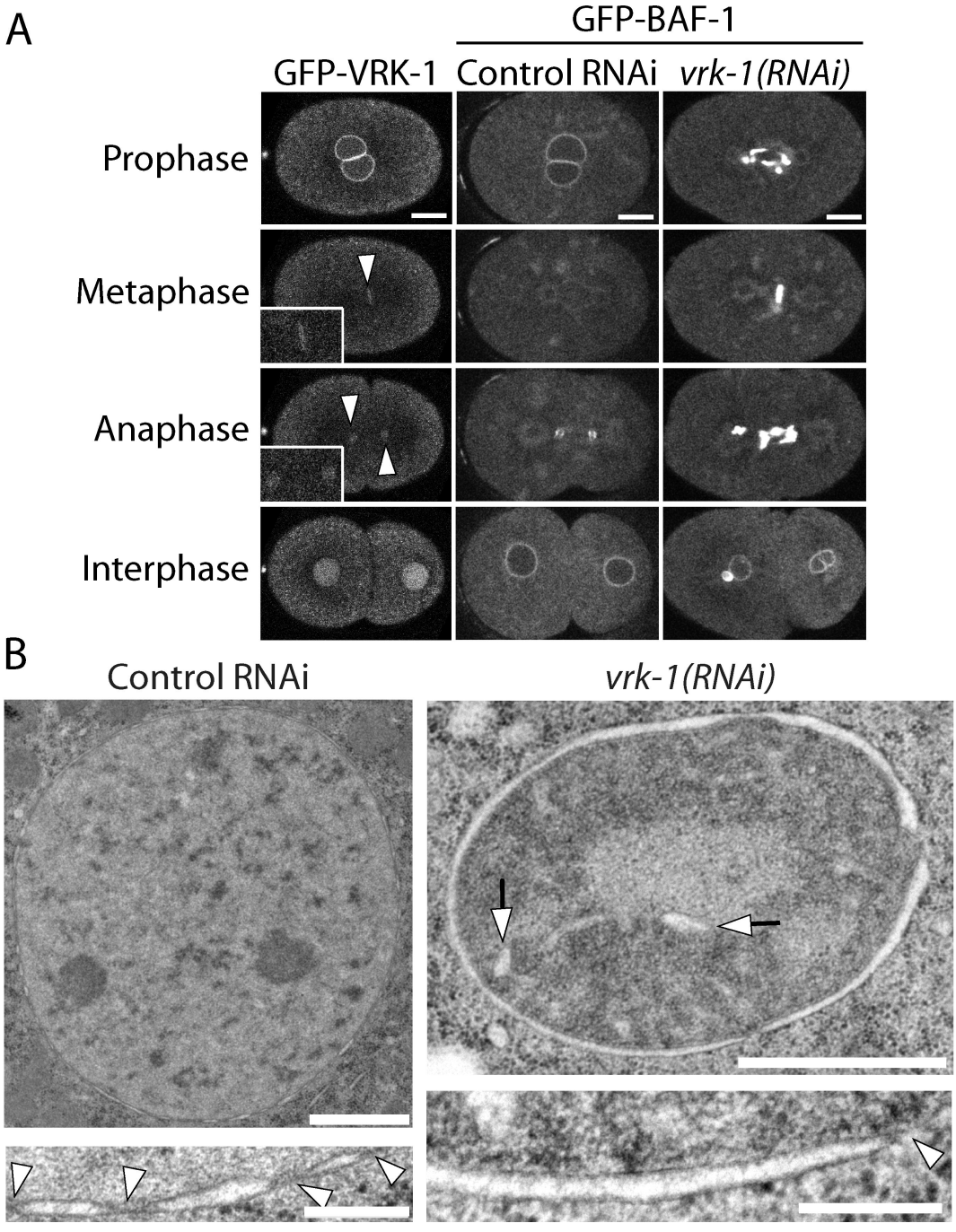

Fig. 2. Dynamic localization of VRK-1 and its target BAF-1. **A.** Frames from time-lapse recordings of the first mitotic division of *C. elegans* embryos expressing GFP-VRK-1 (left column; insert zoom 3x) and GFP-BAF-1 (middle and right columns). GFP-VRK-1 accumulates at the nuclear envelope during prophase immediately before nuclear envelope breakdown. GFP-VRK-1 is chromatin bound during metaphase (arrow) and anaphase (arrows). GFP-BAF-1 is located at the nuclear envelope during interphase and is absent from chromatin during metaphase. Down-regulation of VRK-1 by RNAi (right column) leads to a strong accumulation of GFP-BAF-1 on chromatin during mitosis. Bars: 10 µm. **B.** Transmission electron micrographs of control RNAi and *vrk-1(RNAi)* embryonic nuclei. The nuclear envelope of the control nucleus is gated at regular spaces by nuclear pores (arrowheads). The *vrk-1(RNAi)* nuclei show long stretches of nuclear membrane almost entirely without nuclear pores. In addition, the nuclear size is severely affected and the chromatin is highly condensed and contains intranuclear membranes (arrows). Bars: 1 µm in top panels; 200 nm in bottom panel. Reprinted with permission from (Gorjánácz et al., 2007).

Tenniswood, 2007). p300 and CBP, which acetylate p53 at two different lysine residues, both inhibit p53-mediated downregulation of VRK1, but the effect is independent of their acetyl transferase activity (Valbuena et al., 2008b). This protective effect of both co-activators for VRK1 and for the two VRK2 isoforms, VRK2A and B, is likely a matter of competition for binding to p53, since the C/H3 binding domain of p300 is needed to prevent p53-mediated VRK1 degradation (Valbuena et al., 2008b). VRK2B is thought to be functionally redundant with VRK1 in adenocarcinoma cell lines, in which VRK1 appears mainly cytoplasmic. Although lacking a recognizable NLS, VRK2 enters the nucleus in cells where VRK1 is cytosolic, indicating a certain degree of redundancy in the VRK protein family (Blanco et al., 2006).

In addition to p53, VRK1 has been shown to phosphorylate transcription factors c-Jun and ATF2 (Sevilla et al., 2004a,b). Triggered by numerous cellular stress signals, c-Jun is phosphorylated by multiple MAPKs including ERK and JNK (Chang and Karin, 2001). VRK1 phosphorylates c-Jun in Ser63 and Ser73, resulting in stabilization and intracellular accumulation of c-Jun (Sevilla et al., 2004a). Moreover, phosphorylation of ATF2 Thr73 by VRK1 leads to an increase in ATF2 transcriptional activity (Sevilla et al., 2004b). ATF2 functions in cellular responses mediated by cyclic AMPs, and has been found to form a stable complex with VRK1 (Sevilla et al., 2004b). Transcription of ATF2 is induced by multiple kinases such as JNK, protein kinase A, p38 and VRK1, leading to a complex regulatory network which might secure optimal levels of transcriptionally active ATF2 in cells. The physiological functions of phosphorylation of ATF2 and c-Jun by VRK1 have not been described yet.

Recent data indicate that VRK also regulates cell cycle progression from G1 to S phase by inducing cyclin D1 (CCND1) expression (Kang et al., 2008). From late G1 to early S phase, VRK1 phosphorylates CREB, thereby stimulating CREB's binding to the cAMP response element (CRE) of the CCND1 promoter and activating transcription. In addition, VRK1 modulates association of transcription factor ATF2 with the CRE of CCND1, whereas VRK1 expression is stimulated by Myc binding to its promoter (Kang et al., 2008).

In a manner dependent on cell stimuli, the concerted action of kinases generates a complex pattern of phosphorylation of transcription factors, which are likely to affect their interactions with other proteins and thus transcriptional specificity (Fig. 3). This is a likely explanation for functional differences detected for many transcription factors whose functions vary depending on type of stimulation or environment.

Requirement for VRKs in nuclear envelope dynamics

The nuclear envelope (NE) separates nucleus from cytoplasm and is composed of an outer (ONM) and an

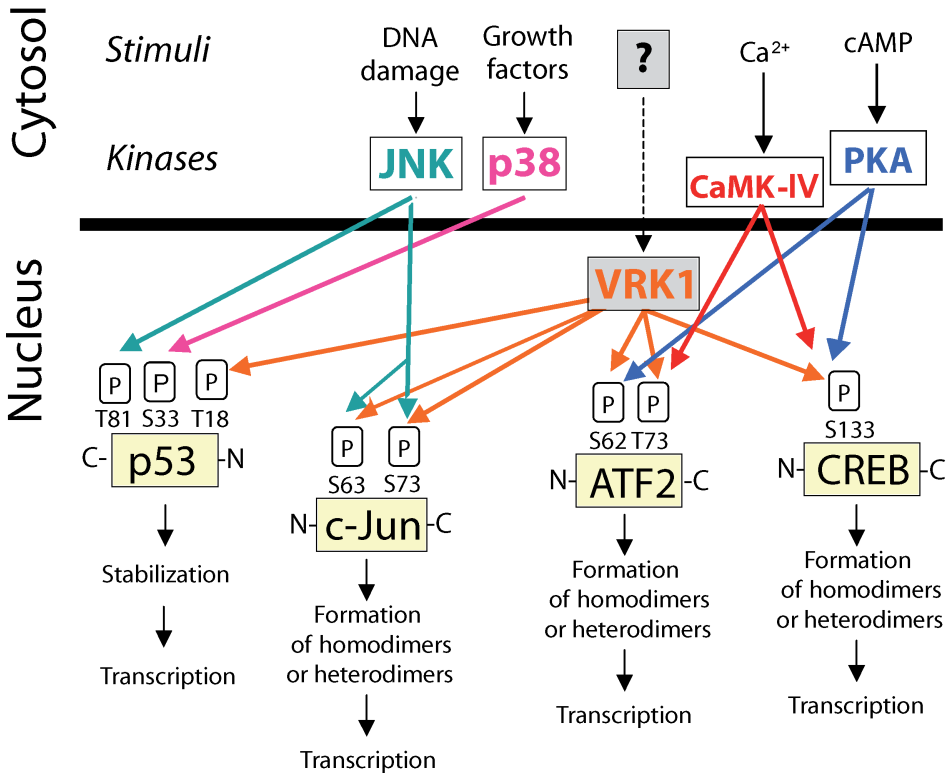

Fig 3. Regulation of transcription factors by VRK1. VRK1 is an important intracellular kinase for activation and/or stabilization of several transcription factors, such as p53, ATF2, c-Jun and CREB. These transcription factors are controlled by a network of protein kinases, phosphorylating different residues in response to various extracellular stimuli. The stimuli to which VRK1 responds are so far unknown. See text for details and references.

inner nuclear membrane (INM) gated at regular distances by nuclear pore complexes (NPCs) allowing controlled transport between the two compartments. We have recently identified the sole *C. elegans* VRK ortholog, CeVRK-1, as an essential regulator of NPC formation (Gorjánácz et al., 2007). Downregulation of CeVRK-1 in *C. elegans* embryos results in an abnormal NE containing nuclear membranes but lacking nuclear pores (Fig. 2B). In *C. elegans* as well as in vertebrates, the small chromatin binding protein, barrier-to-autointegration factor (BAF), is a phosphorylation substrate for VRK (Nichols et al., 2006; Gorjánácz et al, 2007). In addition, a large-scale yeast two-hybrid study of *Drosophila* proteins demonstrated an interaction between the fly homologs of VRK and BAF (Giot et al., 2003). Purified, recombinant VRK1 phosphorylates BAF at its extreme N-terminus on Ser4, and to a lesser extent and slower rate also on Thr2 or Thr3 (Nichols et al., 2006). BAF is associated with chromatin in interphase but is soluble during mitosis (Haraguchi et al., 2001; Gorjánácz et al., 2007) (Fig. 2A). This dynamic behavior is critical for BAF's function in NE assembly and is regulated by the phosphorylation activity of VRK (Nichols et al., 2006; Gorjánácz et al, 2007). As previously mentioned, CeVRK-1 shows a highly dynamic localization pattern (Fig. 2A), which correlates with its phosphorylation of BAF at the NE on entry into mitosis. In the absence of CeVRK-1, BAF is not released from chromatin during mitosis (Fig. 2A), causing a failure in NE breakdown and preventing subsequent postmitotic NE and NPC formation (Gorjánácz et al., 2007).

Recently it was shown that BAF protects cells against vaccinia virus infection. Vaccinia virus, like other poxviruses, replicates its DNA in the cytosol of its host cell. This requires the expression of the B1 kinase, which can be detected immediately after infection (Rempel and Traktman, 1992). At least part of B1's function involves phosphorylation of cytosolic BAF protein to block BAF's ability to bind the viral genome and thereby preventing viral DNA replication (Wiebe and Traktman, 2007). This mechanism thus parallels the requirement of BAF phosphorylation by VRK1 in NE dynamics. Moreover, VRK1 can complement the replication defect of a B1 mutant of vaccinia virus (Boyle and Traktman, 2004), implying that B1 and metazoan VRK kinases not only share structural identity but also functional similarities, albeit in different cell compartments and situations.

Involvement of VRK in the stress response

The cellular stress response involves a wide variety of different mechanisms to respond to various external stresses applied to the cell or organism. Interestingly, recent data have linked the VRK proteins to several types of cellular stress response.

The JNK interacting proteins (JIPs) are a family of scaffold proteins that modulate the MAPKs (Dhanasekaran et al., 2007). VRK2 interacts with JIP1 and modulates the transcriptional responses triggered by interleukin-1ß (IL-1ß) (Blanco et al., 2007, 2008) and hypoxic stress (Blanco et al., 2007). JIP1 assembles a signalosome that recruits phosphorylated JNK, which in turn phosphorylates c-Jun and activates AP1 transcription. Both VRK2 isoforms can form a stable interaction with JIP1, TAK1 and MKK7 and prevent association of JNK with the complex (Blanco et al., 2008). Using a kinase-dead VRK2 mutant it was shown that it is the protein-protein interaction between VRK2 and JIP1, and not the kinase activity of VRK2 that induces a different conformation of the signalosome complex, which is unable to interact with JNK (Blanco et al., 2008).

VRK2 is also able to protect cells from apoptosis by interaction with BHRF1, an Epstein-Barr virus gene product that is homologous to the anti-apoptotic protein Bcl-2 (Li et al., 2006). VRK2 does not interact with Bcl-2 and only possesses mild anti-apoptotic activity itself, but can significantly increase cell survival upon interaction with BHRF1. This interaction is of interest for the development of new cancer treatments, such as targeting epithelial carcinomas that have been linked to infection with Epstein-Barr virus.

Patterning of chromatin modifications by VRK

Posttranslational modifications at tail domains of nucleosomal core histones have emerged as important regulatory mechanism for a multitude of cellular processes (Berger, 2007). Reversible acetylation, phosphorylation, ubiquitination and methylation play essential roles in transcriptional regulation. Histone modifications affect chromatin dynamics and are thought to pattern epigenetic codes that control gene expression. The modifications can both have activating and inactivating effects and are often present together in different combinations, denoted as the 'histone code' (Berger, 2007). For instance, acetylation of histone H3 and H4 residues generally activates gene expression, whereas methylation of H3 and H4 can either repress or induce transcription, depending on the context (Berger, 2007). Phosphorylation of histone H3 Ser10 by multiple kinases is known to play a role in transcription activation and chromosome condensation in both mitosis and meiosis (De Souza et al., 2000; Hsu et al., 2000). In addition, the phosphorylation of histone H2B by the Mst1 kinase is induced in apoptotic cells (Chueng et al., 2003).

Drosophila VRK homolog NHK-1 phosphorylates the conserved Thr119 of histone H2A (Aihara et al., 2004; Ivanovska et al., 2005; Ivanovska and Orr-Weaver, 2006) (Fig. 4). Moreover, this phosphorylation event is required for acetylation of certain lysine residues on histone H3 and H4 (Ivanovska et al., 2005) (Fig. 4). NHK-1 is important for formation and maintenance of the karyosome and metaphase I arrest in meiosis (Ivanovska et al., 2005; Lancaster et al., 2007) and is essential for mitotic progression (Cullen et al., 2005). Mutations in the *nhk-1* gene lead to the formation of a

bipolar metaphase I spindle for each bivalent chromosome, demonstrating that NHK-1 is required to hold the chromosomes and the spindle together during meiosis (Cullen et al., 2005). NHK-1 itself is also phosphorylated in both mitosis and meiosis (Cullen et al., 2005) and its activity is controlled by several other kinases. Aurora B kinase has a positive effect on NHK-1 phosphorylation of histone H2A Thr119 at the centromeric regions during mitosis whilst cyclin B degradation reduces centromeric H2A Thr119 phosphorylation at the onset of anaphase (Brittle et al., 2007). Moreover, in mammals, VRK1 is found to phosphorylate histone H3 on Thr3 and Ser10 during mitosis (Kang et al., 2007). Interestingly, both inactivation of NHK-1 by mutation (Cullen et al., 2005; Ivanovska et al., 2005) or RNAi against CeVRK-1 (Gorjánácz et al., 2007), as well as overexpression of mammalian VRK1, leads to hypercondensed chromatin (Kang et al., 2007). This demonstrates that levels of VRK activity need to be precisely tuned to maintain proper chromatin structure.

Implication of VRK in cell signalling pathways

The positive correlation of VRK1 with proliferation markers such as CDK2, CDK6, and cyclins A and B1 suggested a role for VRK1 in cell cycle progression (Santos et al., 2006). Indeed, VRK1 has been defined as an early response gene in cell cycle progression. More specifically, VRK1 is needed to exit G0 cell cycle phase and enter G1 phase (Valbuena et al., 2008a). RNAi downregulation of VRK1 in cultured cells results in a proliferation block where cells cannot exit G1 phase. VRK1 is expressed simultaneously with early response genes *myc* or *fos*, and precedes cyclin D1 expression. In addition, VRK1 protein level is inversely correlated with p27, an inhibitor of the cell cycle (Valbuena et al., 2008a). The identification of Myc as an activator of VRK1 expression is interesting from a therapeutic point of view, as the Myc signaling pathway is often upregulated in human cancers (Amati et al., 1998; Kang et al., 2008). VRK1 could therefore be targeted as a limiting step in G1/S progression of tumor cells, as suggested by siRNA knockdown of VRK1 (Vega et al., 2004; Valbuena et al., 2008a).

The involvement of VRKs in cell cycle progression is also emerging in *C. elegans*. The germline in *vrk-1* mutants has an apparent cell cycle defect, which results in the inability to form gametes and thus sterility {E.K. & P.A.; unpublished results} (Fig. 5A). A second animal

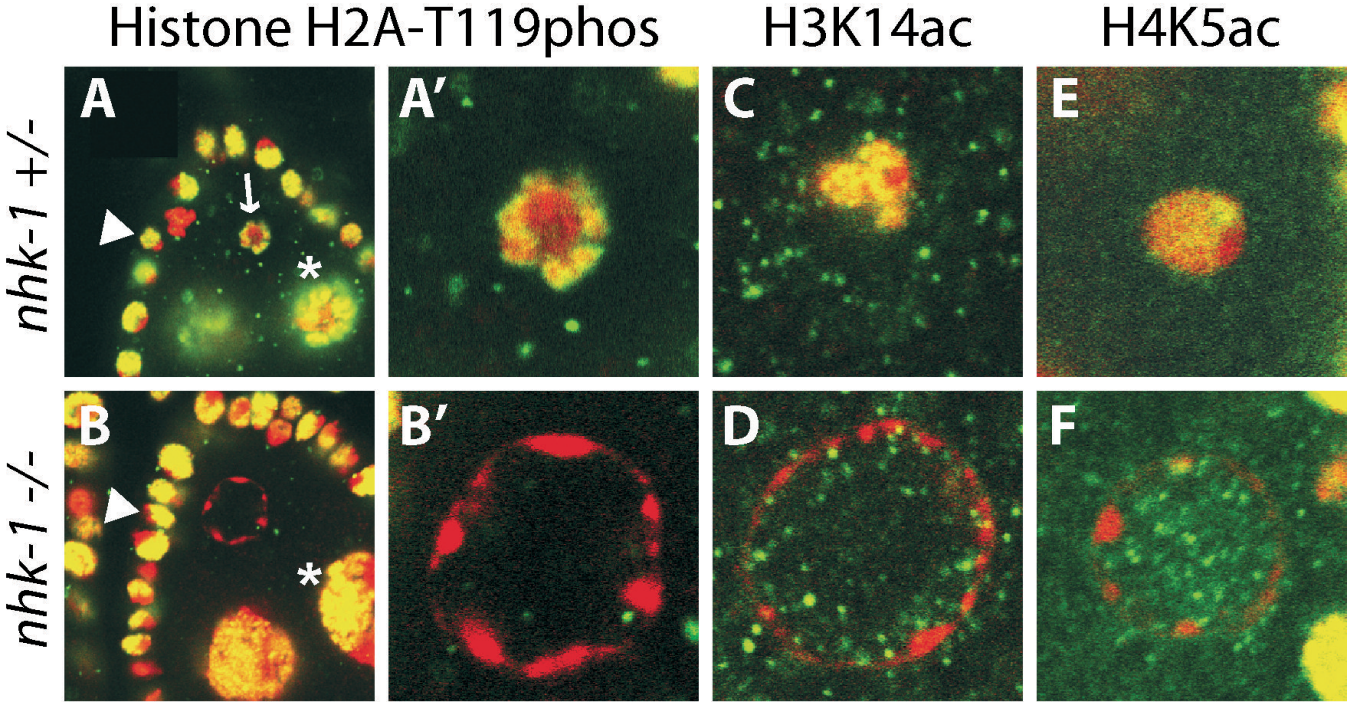

Fig. 4. NHK-1 and the histone code. **A.** Histone H2A-T119phos (green) is present in nurse cells (*), follicle cells (arrowhead), and the karyosome (arrow) in *D. melanogaster nhk-1* heterozygous ovaries, and it colocalizes with the DNA (red). Yellow denotes overlap between the signals. **A'.** The control karyosome at a higher magnification showing the colocalization of H2A-T119phos with the DNA. **B.** H2A-T119phos persists in nurse cells (*) and follicle cells (arrowhead) in the homozygous *nhk-1* mutant ovaries. However, the DNA in the mutant oocyte nucleus lacks H2A-T119phos. **B'.** A higher magnification showing the absence of H2A-T119phos in the mutant oocyte nucleus. **C, E.** Histone H3K14 and histone H4K5 are acetylated in the control karyosomes but not in the *nhk-1* homozygous mutant **(D, F)** (green, H3K14ac **(C, D)** or H4K5ac **(E, F)**; red, DNA; yellow, overlap between signals). Reprinted with permission from (Ivanovska et al., 2005).

model system has also generated a preliminary but very exciting new lead in learning more about the role of VRK proteins in proliferation; mice carrying a mutation in the proliferation of germ cells (*pog*) gene were compared to mice that have a insertional mutation (*gcd*) that knocks down both the *pog* and the *vrk2* gene. Mice homozygous for the *gcd* mutation showed a stronger defect in germ cell proliferation than animals homozygous for the *pog* mutant gene alone (Lu and Bishop, 2003). These results could be interpreted as the *gcd* mutation giving a sensitized background for looking at the *pog* gene, or it is possible that *vrk2* has a function in germ cell development. In support of this, *vrk1*

Table 1. Overview of known VRK phosphorylation substrates.

VRK phosphorylation substrates	
Chromatin associated proteins	BAF (Thr2/3, Ser4) Histone H2A (Thr119) Histone H3 (Thr3, Ser10)
Transcription factors	p53 (Thr18) c-Jun (Ser63, Ser73) ATF2 (Ser62, Thr73) JIP1 CREB (Ser133)

These can be classified as chromatin associated proteins or transcription factors. See text for references.

Fig. 5. Mutation of *vrk-1* leads to sterility. Compared to a wildtype *C. elegans* germline **(A)**, the germline of a *vrk-1* mutant at L4 stage **(B)** contains less and abnormally enlarged germline cells. Development of a normal vulva **(C)** (arrowhead) is impaired in *vrk-1* mutants, leading to a protruding vulva phenotype (pvl) **(D)** (arrowhead). The central vulva precursor cells **(E)** express GFP under control of the EGL-17 FGF promoter (**E'**), a downstream target of the Ras signaling pathway. The EGL-17 promoter is significantly less active in *vrk-1* mutants (**F, F'**).

knockdown mice are viable and show no congenital defects, but become sterile within a few weeks after birth, suggesting a function for *vrk1* in germ cell proliferation (Dr. Valerie Reinke; personal communication).

Of the three vertebrate paralogs of the VRK family, VRK3 is the least characterized to date. mVRK3 interacts with and enhances the activity of vaccinia-H1-related (VHR) phosphatase, an atypical MAPK phosphatase (MKP). This results in increased dephosphorylation of extracellular signal-regulated kinase (ERK) in the nucleus and thus downregulation of the ERK signaling pathway (Kang and Kim, 2006, 2008). Since VRK3 proteins are catalytically inactive as kinases (see above), the regulation of VHR by VRK3 does not involve direct phosphorylation, but rather suggests a novel mechanism of VRK activity. The ERK signaling pathway integrates biological responses and plays a major role in controlling proliferation and differentiation. Tight regulation of its activity is thus essential to ensure normal development. With the discovery of VRK3 as a negative regulator of ERK, VRK3 may potentially be used to develop new therapeutic strategies to address deregulation of ERK in pathologies.

Proteomic analysis has shown that VRK1 and VRK2B interact with the Ras-related Ran GTPase (Sanz-Garcia et al., 2008). Multiple cellular functions for Ran have been described, such as nucleocytoplasmic transport, assembly of the mitotic spindle, chromosome segregation and nuclear envelope formation (Stewart, 2007; Clarke and Zhang, 2008). Binding of RanGDP to VRK1 inhibits VRK1 autophosphorylation and phosphorylation of histone H3 Ser10 and Thr3, but the activity is recovered by interacting with RanGTP (Sanz-Garcia et al., 2008). The biological function of this novel interaction is not known, but association of Ran with VRK1 in the nucleus may be important for VRK1's role in transcription factor regulation. The VRK1-Ran complex also associates with Regulator of Chromosome Condensation, RCC1, suggesting that VRK1's effect on chromatin dynamics may at least in part be mediated via this complex (Sanz-Garcia et al., 2008). These data identify Ran, depending on the bound nucleotide, as the first negative regulator of VRK kinase activity.

As mentioned previously, one of the major cell signaling pathways is the RTK/Ras/MAPK pathway. Critical aspects of this pathway have been delineated by studying vulva development in *C. elegans* (Sternberg, 2005). Because of its relative simplicity – the *C. elegans* vulva consists of 22 cells – organogenesis can be investigated at single-cell resolution, including cell-cell communication among vulva cells and between the vulva and surrounding tissues. Dimerization of the receptor tyrosine kinase LET-23 (EGFR) triggers its autophosphorylation followed by binding of adapter proteins and recruitment of the guanine nucleotide exchange factor SOS-1. SOS-1 loads the small GTPase LET-60/Ras with GTP whereby Ras activates the ERK/MAPK pathway, consisting of the 3 kinases LIN-45 (Raf), MEK-2 (Mek) and MPK-1 (ERK/MAPK). In addition to the Ras signaling cascade, other conserved pathways, such as Wnt or Notch, are involved in vulva development. Perturbation of the balance between these pathways causes a variety of phenotypes, ranging from absence of vulva induction to formation of multiple vulvae. We have recently discovered that mutation in the *C. elegans vrk-1* gene interferes with the RTK/Ras/MAPK pathway and results in highly abnormal vulva morphology (Fig. 5). Determining the exact position of VRK-1 in this signal transduction cascade will be highly relevant not only for understanding how VRK-1 acts in the nematode, but presumably also to elucidating some of VRK's many and interesting functions conserved across species.

Conclusions

Since their initial discovery, the VRKs have gained significant research interest and have been shown to function in a wide variety of biological processes. VRK substrates include various transcription factors, such as p53 and c-Jun and chromatin proteins BAF and histone H2A (Table 1). The diversity of substrates correlates well with VRKs regulating several cellular events, such as cell cycle progression, apoptosis, chromatin organization and nuclear envelope disassembly/assembly cycles. The VRKs have been shown to exert essential functions both by their phosphorylation abilities as protein kinases and by direct protein:protein interaction with different important cellular components. The small GTPase Ran is the first negative regulator described for VRK. The identification of Myc as activator of a putative signaling pathway involved in VRK expression has made VRK a promising candidate for anti-cancer research. Much remains to be learnt about this new emerging kinase family but it is clear that we are gaining new insights into important biological processes with exciting long-term therapeutic potentials.

Acknowledgements. We thank Valerie Reinke and Katherine Waters for sharing unpublished results and Terry Orr-Weaver for Fig. 4. We are grateful to members of the Askjaer laboratory for critical reading and useful comments on the manuscript. Work in the Askjaer laboratory is supported by the Spanish Ministry of Science and Innovation (BFU-2007-60116) and the Andalusian Regional Government (P07-CVI-02697).

References

Aihara H., Nakagawa T., Yasui K., Ohta T., Hirose S., Dhomae N., Takio K., Kaneko M., Takeshima Y., Muramatsu M. and Ito T. (2004). Nucleosomal histone kinase-1 phosphorylates H2A Thr 119 during mitosis in the early *Drosophila* embryo. Genes Dev. 18, 877-888.

Amati B., Alevizopoulos K. and Vlach J. (1998). Myc and the cell cycle. Front. Biosci. 3, 250-268.

Ashcroft M. and Vousden K.H. (1999). Regulation of p53 stability. Oncogene 18, 7637-7643.

Barcia R., López-Borges S., Vega F.M. and Lazo P.A. (2002). Kinetic properties of p53 phosphorylation by the human vaccinia-related kinase 1. Arch. Biochem. Biophys. 399, 1-5.

Bargonetti J. and Manfredi J.J. (2002). Multiple roles of the tumor suppressor p53. Curr. Opin. Oncol. 14, 86-91.

Berger S.L. (2007). The complex language of chromatin regulation during transcription. Nature 447, 407-412.

Blanco S., Klimcakova L., Vega F.M. and Lazo PA. (2006). The subcellular localization of vaccinia-related kinase-2 (VRK2) isoforms determines their different effect on p53 stability in tumour cell lines. FEBS J. 273, 2487-2504.

Blanco S., Santos C. and Lazo P.A. (2007). Vaccinia-related kinase 2 modulates the stress response to hypoxia mediated by TAK1. Mol. Cell. Biol. 27, 7273-7283.

Blanco S., Sanz-García M., Santos C.R. and Lazo P.A. (2008). Modulation of interleukin-1 transcriptional response by the interaction between VRK2 and the JIP1 scaffold protein. PLoS ONE 3, e1660.

Bos J.L. (1989). Ras oncogenes in human cancer: a review. Cancer Res. 49, 4682-4689.

Boyle K.A. and Traktman P. (2004). Members of a novel family of mammalian protein kinases complement the DNA-negative phenotype of a vaccinia virus ts mutant defective in the B1 kinase. J. Virol. 78, 1992-2005.

Brittle A.L., Nanba Y., Ito T. and Ohkura H. (2007). Concerted action of Aurora B, Polo and NHK-1 kinases in centromere-specific histone 2A phosphorylation. Exp. Cell. Res. 313, 2780-2785.

Caenepeel S., Charydczak G., Sudarsanam S., Hunter T. and Manning G. (2004). The mouse kinome: discovery and comparative genomics of all mouse protein kinases. Proc. Natl. Acad. Sci. USA 101, 11707-11712.

Chang L. and Karin M. (2001). Mammalian MAP kinase signalling cascades. Nature 410, 37-40.

Chueng W.L., Ajiro K., Samejima K., Kloc M., Chueng P., Mizzen C.A., Beeser A., Etkin L.D., Chernoff J., Earnshaw W.C. and Allis C.D. (2003). Apoptotic phosphorylation of histone H2B is mediated by mammalian sterile twenty kinase. Cell 113, 507-517.

Clarke P.R. and Zhang C. (2008). Spatial and temporal coordination of mitosis by Ran GTPase. Nat. Rev. Mol. Cell Biol. 9, 464-477.

Cohen P. and Frame S. (2001). The renaissance of GSK3. Nat. Rev. Mol. Cell Biol. 2, 769-776.

Cullen C.F., Brittle A.L., Ito T. and Ohkura H. (2005). The conserved kinase NHK-1 is essential for mitotic progression and unifying acentrosomal meiotic spindles in *Drosophila melanogaster*. J. Cell Biol. 171, 593-602.

De Souza C.P., Osmani A.H., Wu L.P. Spotts J.L. and Osmani S.A. (2000). Mitotic histone H3 phosphorylation by the NIMA kinase in *Aspergillus nidulans*. Cell 102, 293-302.

Dhanasekaran D.N., Kashef K., Lee C.M, Xu H. and Reddy E.P. (2007). Scaffold proteins of MAP-kinase modules. Oncogene 26, 3185-3202.

Dornan D., Wertz I., Shimizu H., Arnott D., Frantz G.D., Dowd P., O'Rourke K., Koeppen H. and Dixit V.M. (2004). The ubiquitin ligase COP1 is a critical negative regulator of p53. Nature 429, 86-92.

Giot L., Bader J.S., Brouwer C., Chaudhuri A., Kuang B., Li Y., Hao Y.L., Ooi C.E., Godwin B., Vitols E., Vijayadamodar G., Pochart P., Machineni H., Welsh M., Kong Y., Zerhusen B., Malcolm R., Varrone Z., Collis A., Minto M., Burgess S., McDaniel L., Stimpson E., Spriggs F., Williams J., Neurath K., Ioime N., Agee M., Voss E., Furtak K., Renzulli R., Aanensen N., Carrolla S., Bickelhaupt E., Lazovatsky Y., DaSilva A., Zhong J., Stanyon C.A., Finley R.L. Jr., White K.P., Braverman M., Jarvie T., Gold S., Leach M., Knight J., Shimkets R.A., McKenna M.P., Chant J. and Rothberg J.M. (2003). A protein interaction map of *Drosophila melanogaster*. Science 302, 1727-1736.

Gorjánácz M., Klerkx E.P.F., Galy V., Santarella R., Lopez-Iglesias C., Askjaer P. and Mattaj I.W. (2007). *Caenorhabditis elegans* BAF-1 and its kinase VRK-1 participate directly in post-mitotic nuclear envelope assembly. EMBO J. 26, 132-143.

Hanks S.K. and Hunter T. (1995). Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. FASEB J. 9, 576-596.

Haraguchi T., Koujin T., Segura-Totten M., Lee K.K., Matsuoka Y, Yoneda Y., Wilson K.L. and Hiroaka Y. (2001). BAF is required for emerin assembly into the reforming nuclear envelope. J. Cell Sci. 114, 4575-4585.

Hsu J.Y., Sun Z.W., Li X., Reuben M., Tatchell K., Bishop D.K., Grushcow J.M., Brame C.J., Caldwell J.A., Hunt D.F., Lin R., Smith M.M. and Allis C.D. (2000). Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes. Cell 102, 279-291.

Ivanovska I. and Orr-Weaver T.L. (2006). Histone modifications and the chromatin scaffold for meiotic chromosome architecture. Cell Cycle 5, 2064-2071.

Ivanovska I., Khandan T., Ito T. and Orr-Weaver T.L. (2005). A histone code in meiosis: the histone kinase, NHK-1, is required for proper chromosomal architecture in Drosophila oocytes. Genes Dev. 19, 2571-2582.

Kang T.H. and Kim K.T. (2006). Negative regulation of ERK activity by VRK3-mediated activation of VHR phosphatase. Nat. Cell Biol. 8, 863-869.

Kang T.H. and Kim K.T. (2008). VRK3-mediated inactivation of ERK signaling in adult and embryonic rodent tissues. Biochim. Biophys. Acta 1783, 49-58.

Kang T.H., Park D.Y., Choi Y.H., Kim K.J., Yoon H.S. and Kim K.T. (2007). Mitotic histone H3 phosphorylation by vaccinia-related kinase 1 in mammalian cells. Mol. Cell. Biol. 27, 8533-8546.

Kang T.H., Park D.Y., Kim W. and Kim K.T. (2008). VRK1 phosphorylates CREB and mediates CCND1 expression. J. Cell. Sci. 121, 3035-3041.

Karnoub A.E. and Weinberg R.A. (2008). Ras oncogenes: split personalities. Nat. Rev. Mol. Cell Biol. 9, 517-531.

Lancaster O.M., Cullen C.F. and Ohkura H. (2007). NHK-1 phosphorylates BAF to allow karyosome formation in the *Drosophila* oocyte nucleus. J. Cell Biol. 179, 817-824.

Leng R.P., Lin Y., Ma W., Wu H., Lemmers B., Chung S., Parant J.M., Lozano G., Hakem R. and Benchimol S. (2003). Pirh2, p53-induced ubiquitin-protein ligase, promotes p53 degradation. Cell 112, 779-791.

Li M., Brooks C.L., Kon N. and Gu W. (2004). A dynamic role of HAUSP in the p53-Mdm2 pathway. Mol. Cell 13, 879-886.

Li L.Y., Liu M.Y., Shih H.M., Tsai C.H. and Chen J.Y. (2006). Human cellular protein VRK2 interacts specifically with Epstein-Barr virus BHRF1, a homologue of Bcl-2, and enhances cell survival. J. Gen. Virol. 87, 2869-2878.

López-Borges S. and Lazo P.A. (2000). The human vaccinia-related kinase 1 (VRK1) phosphorylates threonine-18 within the mdm-2

binding site of the p53 tumour suppressor protein. Oncogene 19, 3656-3664.

Lu B. and Bishop C.E. (2003). Late onset of spermatogenesis and gain of fertility in POG-deficient mice indicate that POG is not necessary for the proliferation of spermatogonia. Biol. Reprod. 69, 161-168.

Manning G., Plowman G.D., Hunter T. and Sudarsanam S. (2002). Evolution of protein kinase signaling from yeast to man. Trends Biochem. Sci. 27, 514-520.

McKay M.M. and Morrison D.K. (2007). Integrating signals from RTKs to ERK/MAPK. Oncogene 26, 3113-3121.

Meek D.W. (1999). Mechanisms of switching on p53: a role for covalent modification? Oncogene 18, 7666-7675.

Nezu J., Oku A., Jones M.H. and Shimane M. (1997). Identification of two novel human putative serine/threonine kinases, VRK1 and VRK2, with structural similarity to vaccinia virus B1R kinase. Genomics 45, 327-331.

Nichols R.J. and Traktman P. (2004). Characterization of three paralogous members of the Mammalian vaccinia related kinase family. J. Biol. Chem. 279, 7934-7946.

Nichols R.J., Wiebe M.S. and Traktman P. (2006). The vaccinia-related kinases phosphorylate the N' terminus of BAF, regulating its interaction with DNA and its retention in the nucleus. Mol. Biol. Cell. 17, 2451-2464.

Raman M., Chen W. and Cobb M.H. (2007). Differential regulation and properties of MAPKs. Oncogene 26, 3100-3112.

Rempel R.E. and Traktman P. (1992). Vaccinia virus B1 kinase: phenotypic analysis of temperature-sensitive mutants and enzymatic characterization of recombinant proteins. J. Virol. 66, 4413-4426.

Roy S. and Tenniswood M. (2007). Site specific acetylation of p53 directs selective transcription complex assembly. J. Biol. Chem. 282, 4765-4771.

Ryan K.M., Phillips A.C. and Vousden K.H. (2001). Regulation and function of the p53 tumor suppressor protein. Curr. Opin. Cell Biol. 13, 332-337.

Santos C.R., Rodríguez-Pinilla M., Vega F.M., Rodríguez-Peralto J.L., Blanco S., Sevilla A., Valbuena A., Hernández T., van Wijnen A.J., Li F., de Alava E., Sánchez-Céspedes M. and Lazo P.A. (2006). VRK1 signaling pathway in the context of the proliferation phenotype in head and neck squamous cell carcinoma. Mol. Cancer Res. 4, 177-185.

Sanz-García M., López-Sánchez I. and Lazo P.A. (2008). Proteomics identification of nuclear Ran GTPase as an inhibitor of human VRK1 and VRK2 (vaccinia-related kinase) activities. Mol. Cell Proteomics 7, 2199-2214.

Sevilla A., Santos C.R., Barcia R., Vega F.M. and Lazo P.A. (2004a). c-Jun phosphorylation by the human vaccinia-related kinase 1 (VRK1) and its cooperation with the N-terminal kinase of c-Jun (JNK). Oncogene 23, 8950-8958.

Sevilla A., Santos C.R., Vega F.M. and Lazo P.A. (2004b). Human vaccinia-related kinase 1 (VRK1) activates the ATF2 transcriptional activity by novel phosphorylation on Thr-73 and Ser-62 and cooperates with JNK. J. Biol. Chem. 279, 27458-27465.

Stad R., Little N.A., Xirodimas D.P., Frenk R., van der Eb A.J., Lane D.P., Saville M.K. and Jochemsen A.G. (2001). Mdmx stabilizes p53 and Mdm2 via two distinct mechanisms. EMBO Rep. 2, 1029-1034.

Stewart M. (2007). Molecular mechanism of the nuclear protein import cycle. Nat. Rev. Mol. Cell Biol. 8, 195-208.

Sternberg, P.W. (2005). Vulval development. WormBook, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.6.1, http://www.wormbook.org.

Sundaram M.V. (2006). RTK/Ras/MAPK signaling. WormBook., ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.80.1. http://www.wormbook.org.

Toledo F. and Wahl G.M. (2006). Regulating the p53 pathway: *in vitro* hypotheses, *in vivo* veritas. Nat. Rev. Cancer 6, 909-923.

Traktman P., Anderson M.K. and Rempel R.E. (1989). Vaccinia virus encodes an essential gene with strong homology to protein kinases. J. Biol. Chem. 264, 21458-21461.

Valbuena A., Vega F.M., Blanco S. and Lazo P.A. (2006). p53 downregulates its activating vaccinia-related kinase 1, forming a new autoregulatory loop. Mol. Cell. Biol. 26, 4782-4793.

Valbuena A., López-Sánchez I., Vega F.M., Sevilla A., Sanz-García M., Blanco S. and Lazo P.A. (2007a). Identification of a dominant epitope in human vaccinia-related kinase 1 (VRK1) and detection of different intracellular subpopulations. Arch. Biochem. Biophys. 465, 219-226.

Valbuena A., Suárez-Gauthier A., López-Rios F., López-Encuentra A., Blanco S., Fernández P.L., Sánchez-Céspedes M. and Lazo P.A. (2007b). Alteration of the VRK1-p53 autoregulatory loop in human lung carcinomas. Lung Cancer 58, 303-309.

Valbuena A., López-Sánchez I. and Lazo P.A. (2008a). Human VRK1 is an early response gene and its loss causes a block in cell cycle progression. PLoS ONE 3, e1642.

Valbuena A., Blanco S., Vega F.M. and Lazo P.A. (2008b). The C/H3 domain of p300 is required to protect VRK1 and VRK2 from their downregulation induced by p53. PLoS ONE 3, e2649.

Vega F.M., Gonzalo P., Gaspar M.L. and Lazo P.A. (2003). Expression of the VRK (vaccinia-related kinase) gene family of p53 regulators in murine hematopoietic development. FEBS Lett. 544, 176-180.

Vega F.M., Sevilla A. and Lazo P.A. (2004). p53 Stabilization and accumulation induced by human vaccinia-related kinase 1. Mol. Cell. Biol. 24, 10366-10380.

Vousden K.H. and Lu X. (2002). Live or let die: the cell's response to p53. Nat. Rev. Cancer 2, 594-604.

Wiebe M.S. and Traktman P. (2007). Poxviral B1 kinase overcomes barrier to autointegration factor, a host defense against virus replication. Cell Host Microbe 1, 187-197.

Yuan Z.M., Huang Y., Ishiko T., Nakada S., Utsugisawa T., Shioya H., Utsugisawa Y., Yokoyama K., Weichselbaum R., Shi Y. and Kufe D. (1999). Role for p300 in stabilization of p53 in the response to DNA damage. J. Biol. Chem. 274, 1883-1886.

Zelko I., Kobayashi R., Honkakoski P. and Negishi M. (1998). Molecular cloning and characterization of a novel nuclear protein kinase in mice. Arch. Biochem. Biophys. 352, 31-36.

Accepted December 10, 2008