

Nuclear compartmentation of HIPK2, a kinase with many talents

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The nucleus is highly structured and a complex nuclear architecture ensures the coordinated function of nuclear processes including transcription, splicing, replication, DNA repair, ribosome assembly and the assurance of the proper allocation of genetic and epigenetic information (Cremer and Cremer, 2001; Lamond and Spector, 2003). These processes occur in subnuclear structures lacking a delineating membrane and are collectively called nuclear speckles including Cajal bodies, PML (promyelocytic leukemia) nuclear bodies (PML-NBs), splicing factor compartments and polycomb nuclear bodies, just to name a few (Leonhardt and Cardoso, 2000). This ever-growing list of nuclear speckles also includes HIPK domains, which contain the name-giving serine/threonine kinase HIPK2 (homeodomain interacting protein kinase 2) and HIPK3 proteins (Möller et al., 2003). HIPK bodies display a variable size between 0.1 and 1 μ M and are found throughout the nucleus with a tendency to occur with an increased frequency in the nuclear periphery (Fig. 1). The kinase function of HIPK2 is essential for its localization to speckles, while a kinase inactive point mutant localizes throughout the nucleoplasm. The phosphorylation substrate allowing HIPK domain localization remains to be identified in the future. HIPK2 is the founding member of the HIPK family which includes HIPK1, HIPK2

and HIPK3. These three kinases are very homologous to each other, raising the possibility that they are functionally redundant. Murine HIPK2 was cloned as an interactor of the homeodomain transcription factor NKx-1.2 in a yeast two-hybrid screen (Kim et al., 1998). HIPK2 proteins were also cloned from hamster (termed PKM) (Trost et al., 2000) and human (Hofmann et al., 2000; Wang et al., 2001). HIPK2 can be sumoylated at the N-terminal lysine 25, followed by a kinase domain, an interaction domain (ID) for the homeodomain transcription factors, a corepressor domain, two putative PEST motifs (rich in Proline, Glutamic acid, Serine and Threonine) and a tyrosine/histidine-rich motif (YH) at the C-terminus. A speckle retention signal (SRS) was mapped for the murine HIPK2 to 860-967aa (Kim et al., 1999). The kinase domain displays a remarkable degree of evolutionary conservation with orthologs occurring even in *Drosophila melanogaster* and *Caenorhabditis elegans* (Fig. 2). Ongoing work in our laboratory focuses on understanding the molecular mechanisms and signaling pathways mediating the variety of cellular functions of this ancient protein kinase.

HIPK2 as a transcriptional repressor

HIPK2 enhances the DNA binding activity of the NK-3 homeoprotein transcription factor

and greatly potentiates its repressor activity. Furthermore, it can also bind to further members of the homeodomain family such as NK-1, NKx-2.5, HoxC4 and HoxD4, but the physiological meaning of these interactions remains to be studied (Kim et al., 1998). HIPK2 also interacts with TTF-1 (also named Nkx2.1 or thyroid transcription factor-1), a member of Nkx2 class of homeodomain-containing proteins. TTF-1/Nkx2.1 is involved in the regulation of thyroid, lung, and ventral forebrain development. Conversely HIPK2 displays no effect on TTF-1 transcriptional activity in either thyroid or HeLa cells; thus, the functional role of this interaction remains to be established. In addition, HIPK2 was found as part of a corepressor complex containing Groucho, mSin3A and a histone deacetylase where HIPK2 may play a role in the corepressor complex formation (Choi et al., 1999). Further evidence for a potential role of HIPK2 in transcriptional repression comes from the recently discovered interaction with the transcriptional corepressor CtBP (carboxyl-terminal binding protein). In response to DNA damage, HIPK2 phosphorylates CtBP at serine 422, thus resulting in subsequent proteasome dependent elimination of CtBP. Proteolytic degradation of CtBP by HIPK2 eliminates its antiapoptotic function and thus promotes apoptosis (Zhang et al., 2003). HIPK2 is also required for the c-Ski-mediated inhibition of Smad1/4-dependent transactivation in a BPM (bone morphogenetic protein)-induced signaling pathway. HIPK2 binds to both Smad1 and c-Ski and colocalizes with these proteins in HIPK domains (Harada et al., 2003). HIPK2 functions as a transcriptional corepressor that downregulates Brn3a-mediated gene expression of brn3a, trkA and bcl-xL, which are known for their anti-apoptotic function (Wiggins et al., 2004). Elimination of these anti-apoptotic proteins by HIPK2 overexpression induces apoptosis in cultured sensory neurons, as well as in developing neurotrophin-dependent sensory and sympathetic neurons (Wiggins et al., 2004). Conversely, targeted disruption of HIPK2 leads to increased expression of Brn3a, TrkA, and Bcl-xL and consequently to reduced apoptosis. Collectively, these data

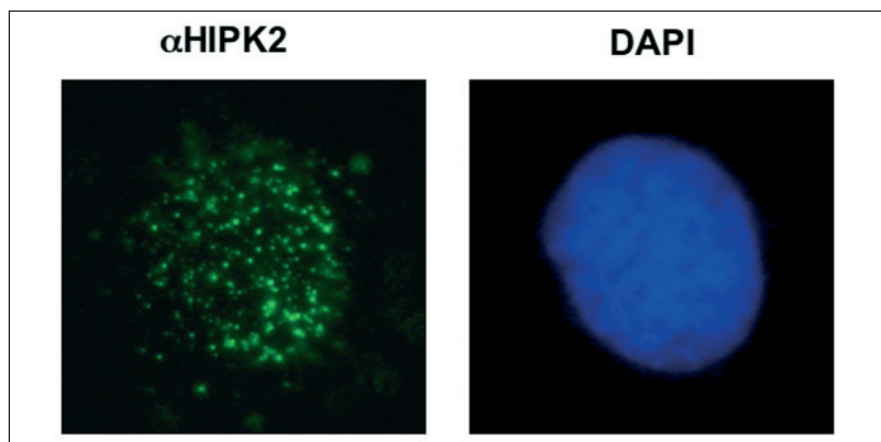


Fig. 1: Intracellular localization of HIPK2. U2OS cells were stained with α HIPK2 antibodies to reveal the localization of the endogenous kinase by indirect immunofluorescence. DAPI-stained chromosomal DNA is shown at the right.

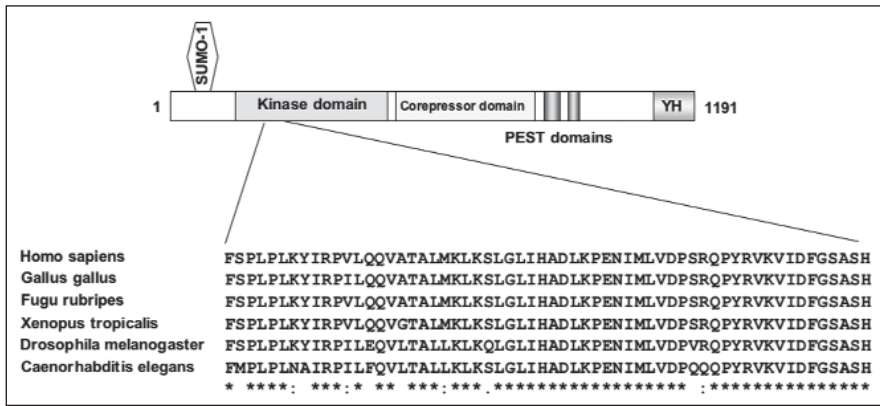


Fig. 2: Architecture and evolutionary conservation of HIPK2. Schematic representation of HIPK2, the various domains are indicated by shading. The lower part shows a sequence comparison of HIPK2 proteins from the indicated species. HIPK2 proteins were aligned using the Clustalw program, identical amino acids are marked by stars, homologous and related amino acids are indicated.

indicate an important role for HIPK2 in transcriptional silencing.

p53-dependent regulation of cell cycle arrest and apoptosis by HIPK2

In addition to its function within transcription repressor complexes, HIPK2 activation by DNA damage leads to cell cycle arrest and apoptosis (Pierantoni et al., 2001; Hofmann et al., 2002; D'Orazi et al., 2002). These effects are mediated by p53-dependent and -independent pathways. HIPK2 and HIPK1 were found as interactors of p53 (Hofmann et al., 2002; D'Orazi et al., 2002; Kondo et al., 2003) and HIPK2 can physically and functionally interact with the p53 family member p73 (Kim et al., 2002). In response to DNA damage, HIPK2 phosphorylates p53 at serine 46 (Hofmann et al., 2002; D'Orazi et al., 2002). This phosphorylation site is critical for the decision whether p53 activation results in growth arrest and subsequent DNA repair or alternatively -when the severity of DNA damage exceeds the capacity of repair mechanisms- in apoptosis (Oda et al., 2000). Phosphorylation of p53 at serine 46 allows subsequent CBP (CREB-binding protein)-mediated p53 acetylation at lysine 382, thus increasing p53-dependent gene expression and promoting apoptosis. HIPK domains show only a partially overlapping localization with PML-NBs, but colocalization of HIPK2 and p53/p73 and phosphorylation of p53 at serine 46 occurs only in PML-NBs (Möller et al., 2003). But not only phosphorylation of p53 takes place in PML-NBs, as also CBP (CREB binding protein)-mediated acetylation and HAUSP-dependent deubiquitination of p53 occur in PML-NBs leading to the suggestion that these subnuclear structures function as molecular hubs (Bernardi and Pandolfi, 2003). Only overexpression of the PML isoform PML-IV causes complete recruitment of HIPK2 to

PML-NBs (Bernardi and Pandolfi, 2003), while no recruitment occurs after expression of PML-III which lacks the C-terminal exon 8 contained in PML-IV. The molecular mechanisms mediating this recruitment remain to be elucidated in future studies, but presumably involve an interaction between PML and HIPK2. There is recent evidence that p53 serine 46 phosphorylation also involves axin,

which can bind to HIPK2 and also p53. The stimulatory effect of Axin on p53-dependent transcriptional activity is abrogated in the presence of kinase inactive HIPK2, indicating that Axin stimulates p53 function through HIPK2 kinase activity. Removal of Axin-interacting domain from HIPK2 (HIPK2 Δ Axin) stimulates p53 transactivation even more dramatically than wild type HIPK2, suggesting that Axin stimulates HIPK2 by physically occupying an autoinhibitory domain of HIPK2 (Rui et al., 2004). HIPK2 also positively regulates the p53 level upon decreasing the amount of Mdm2 protein (Wang et al., 2001). In addition, HIPK2 antagonizes Mdm2-mediated nuclear export and ubiquitination of p53 (Di Stefano et al., 2004), but the molecular mechanism mediating these effects remains to be worked out in the future.

p53-independent cell cycle arrest and apoptosis by HIPK2

There is also ample evidence for p53-independent apoptotic pathways triggered by HIPK2. For example, HIPK2 and Daxx cooperate for the induction of TGF β -induced apoptosis in human p53-deficient hepatocellular carcinoma cells (Hofmann et al., 2003). HIPK2 inter-

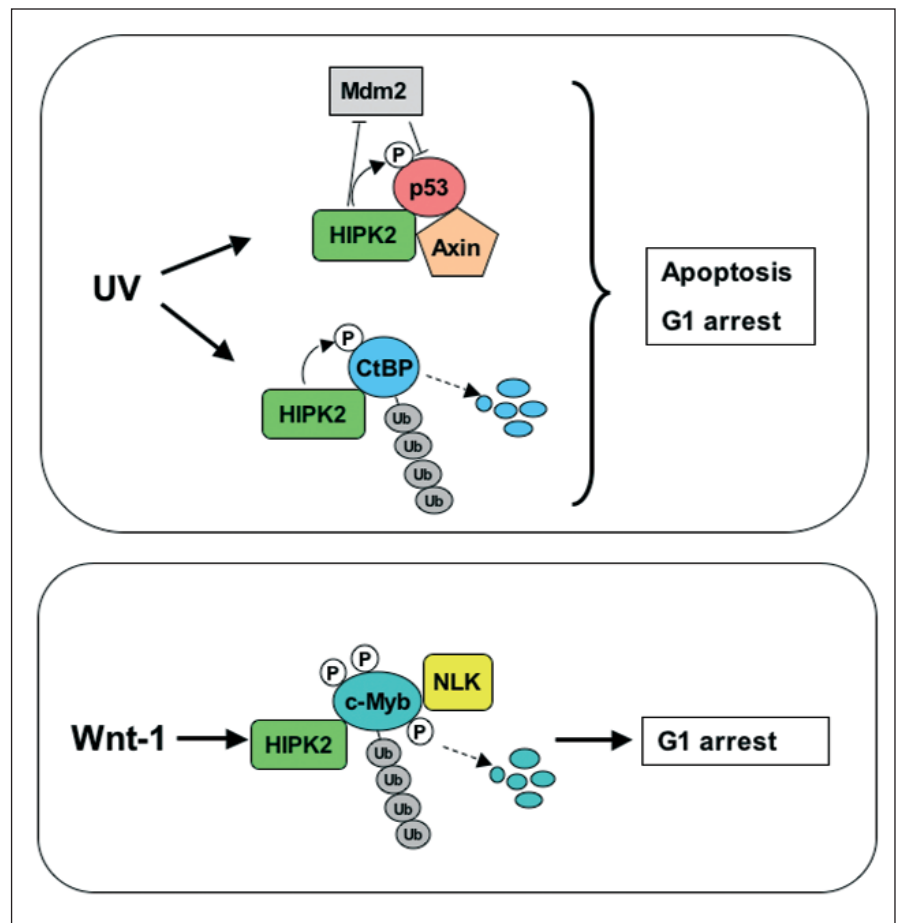


Fig. 3: Summary of HIPK2 signaling pathways. For further details, see text.

acts with its phosphorylation substrate Daxx and synergizes with Daxx to trigger the JNK signaling pathway via the mitogen-activated protein kinase kinases MKK4/SEK1 and MKK7 (Hofmann et al., 2003). HIPK2 is also a member of the Wnt signaling pathway. Nuclear entry of TGF β -activated kinase 1 (TAK1) in response to Wnt signaling leads to the activation of HIPK2 and Nemo-like kinase (NLK). HIPK2 and NLK directly bind to c-Myb and cause its phosphorylation at multiple sites, thus allowing subsequent proteasome-dependent degradation of c-Myb (Kanei-Ishii et al., 2004). Mutation of three putative c-Myb phosphorylation sites prevents HIPK2/NLK-induced proteolytic degradation, thus providing evidence that the direct phosphorylation of c-Myb is required for its subsequent degradation. This in turn inhibits c-Myb-dependent activation of the c-myc promoter and thus induces G1 arrest. Constitutive expression of c-Myb blocks IL-6-induced differentiation of the myeloblastic cell line M1. Overexpression of NLK causes c-Myb degradation which then allows IL-6-induced differentiation of myeloblastic cells, revealing the relevance of TAK1/HIPK2/NLK signaling for the differentiation of myeloblastic cells (Kanei-Ishii et al., 2004). As Wnt signaling also modulates the diversification of hematopoietic cells and self-renewal of hematopoietic stem cells (Staal and Clevers, 2005), it will be interesting to see whether these processes are also regulated by HIPK2. The functional roles of HIPK2 in the regulation of Wnt signaling and cell cycle arrest are schematically summarized in Fig. 3. How can a single kinase fulfil so many different functions? We speculate that part of the answer is the highly structured localisation of HIPK2 in subnuclear domains. While the HIPK2 fraction contained in PML-NBs can be active as a kinase phosphorylating p53 and probably also further PML-NB resident proteins, other localisations in the nucleoplasm or HIPK domains allow to meet further substrate proteins and to mediate additional functions. Even a potential function of HIPK2 in the cytosol cannot be excluded, as the nucleus is disrupted during mitosis. Another layer of complexity is added by the fact that HIPK2 occurs in many different splice variants, which all might display distinct functions. The combinatorial use of modern approaches from cell biology, biochemistry and genetics will help to unravel further secrets of this ancient protein kinase.

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