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**“Stop Ne(c)king around”: How interactomics contributes to functionally characterizeNek family kinases**

Meirelles GV *et al.* Nek family kinase interactomes and functions

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**Abstract**

Aside from Polo and Aurora a third but less studied kinase family involved in mitosis regulation are the Neks (NIMA-related kinases). The founding member of this family is the sole member NIMA (never in mitosis-gene A) of *Aspergillus nidulans*, which is crucial for the initiation of mitosis in that organism. All 11 human Neks have been functionally assigned to one of the three core functions in mammals, established for this family: (1) centrioles/mitosis; (2) primary ciliary function/ciliopathies; and (3) DNA damage response (DDR). Recent findings, especially on Nek 1 and 8, showed however, that several Neks participate in parallel in at least two of the contexts: in this case primary ciliary function and DDR. In the core section of this in depth review we report the current detailed functional knowledge on each of the 11 Neks. In the discussion, we return to the cross-connections among Neks and point out how our and other groups functional and interactomics studies revealed that most Neks interact with protein partners associated with two if not all three of the functional contexts. We then raise the hypothesis that Neks may be the connecting regulatory elements that allow the cell to fine tune and synchronize the cellular events associated with these three core functions. The new and exciting findings on the Nek family open new perspectives and should allow the Neks to finally claim the attention they deserve in the field of kinases and cell cycle biology.

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**Core tip:** NIMA-related kinases (Neks) are a family of 11 human kinases involved in cell cycle regulation. This article represents an in-depth review of the current knowledge on the function of each of the 11 human Nek kinases. Furthermore, we represent arguments in the discussion, how systems biology, especially interactomics, helped to uncover that the majority of Neks are involved in more than one of the three Neks core functions: (1) centrioles/mitosis; (2) primary ciliary function/ciliopathies; and (3) the DNA damage response. Possibly, the Neks act on a higher regulatory level which may control the core functions.

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**INTRODUCTION**

The NIMA-related kinases (Neks) represent aside from Polo and Aurora kinase families a third family of mitotic kinases, but remain to date the least studied and henceforth least understood family of kinases involved in the regulation of the cell cycle. The founding member of this family of kinases is the *Aspergillus nidulans* NimA (Never in mitosis-gene A), which exists as a single member in this fungus and is functionally involved in the initiation of mitosis and promotes the chromosome condensation by phosphorylation of histone H3[1]. In humans we have 11 members of the Nek family, which show highly conserved kinase domains, but differ significantly in the composition and length of their N- and specially C-terminal regulatory and docking domains (Figure 1).

Although some protein interaction partners have been described for the majority of the human Neks (Figure 2), the domain of interaction at the side of Neks has been mapped only for a smaller subset of interacting proteins (Figure1). As we can see, most interactors are assigned to specific regions in the regulatory domains, which represent in most cases classical protein-protein interaction modules, such as coiledcoil regions. Identification of interaction with the kinases domains have been scarce, due to the transient and weak nature of these interactions and therefore the discovery and characterization of true *bona fidein vivo* substrates of Nek kinases remains one of the main challenges in the field. Among the interacting proteins identified by our[2,3] and other groups, through both yeast two-hybrid screens and mass spectrometry analyses,there were hopefully not only those that regulate the Neks but maybe also candidate substrate proteins. The binding of these substrate proteins possibly contributes to “opening up” the Neks orto the activation of these kinases and then, as a consequence, these proteins may be phosphorylated by the Neks.

There has been a series of very good and concise reviews on NIMA and Neks in the past years[4-8]. However, due to scarce or absent knowledge on several family members, including Nek5, 10 and 11 for instance, most reviews opted to focus on a subset of Neks or grouped them according to phylogenetic or functional relatedness. Here, we try to discuss all of the 11 human Neks in some depthand to include all recent novelty on the least studied Neks as well as our own groups published and non-published findings with a special emphasis on the characterization of the functional context based on the identification of interacting proteins (interactomics). A point we like to stress here is that most Neks interact with proteins of several of the classical functional contexts reported initially for a subset of specific Neks. In other words, we may characterize as the main functional contexts of Neks the following three areas: (1) centriolar function and mitosis regulation (Nek2, 6, 7 and 9); (2) primary ciliary function, ciliopathies and microtubule dynamics in general (Nek1, 4 and 8); and more recently; and (3) DDR and G2/M checkpoint (Nek1, 4, 6, 8, 10 and 11)[8, 9].

 However, published interactome data (Figure 2), as well as our groups efforts to identify new interacting proteins for all Neks, showed some surprising cross-connections and novelties, which we would like to point out here. Most of the above mentioned Neks seem to interact with proteins that are functionally linked to two or even all three of the above mentioned areas, raising thereby the possibility that these are somehow connected on a higher regulatory level and that the Neks may be key elements to understand how the regulation of these functional contexts is performed. A typical recently published example is the role of Nek8 in both primary ciliary function and DNA repair mechanisms[10]. Our own studies revealed that Nek6, a kinase primarily associated to mitotic regulatory events[11,12] interacts also with proteins involved in the DNA damage response such as Putative DNA repair and recombination protein RAD26-like (RAD26L) and PHD finger protein 1 (PHF1) (Figure 2)[3]. In fact for the majority of Neks we found interacting partners of the DDR or effector proteins of different DNA repair pathways, which clearly suggests a larger than initially imagined involvement of Neks in this biological processes. Other insights came from the identification of interacting proteins from the apoptosis regulatory pathways with several Neks (*e.g.,* Nek1[13] and 5). This suggests that-aside the well established mitotic context–we must be open minded for additional new roles for Neks. Before we go into details of new cross-connections and suggested additional functional contexts in the final discussion, we will present each of the 11 human Neks in detail in the following section of this review.

**NEK1**

Although Nek1is after Nek2 and aside Nek6 only the third most studied Nek family member, it is in many ways arepresentative member of this family of protein kinases. Along this line, Nek1 started to draw attention of the kinase and signaling research communities not only to itself but to the Nek family after the publication of the seminal article of Upadhya and co-workers in 2000[14]. It reported that deletion mutations in the *Nek1* gene in mice caused polycystic kidney disease (PKD) among other pleiotropic effects, ranging from facial dysmorphism, dwarfing, male sterility, anemia and cystic choroid plexus. The pleiotropic nature of these phenotypes suggested early on a role of Nek1 in basic cellular functions, possibly involved in signaling pathways associated to polycystin-1 and -2, whose mutations also cause PKD and which signaling initiates at the renal epithelial cell’s primary cilia[15].

 Recently, another set of insertion, non-sense and splice site mutations in the *Nek1* gene have been reported in Majewski type short-rib polydactyl syndrome (SRPS), an autosomal-recessive familiar ciliopathy[16,17]. Ciliopathies have been associated with a series of defects of proteins involved in intra-flagellar transport (IFT), as well as cilia, basal body and centrosome maintenance and, in the case of Nek1, SRPS also presents a broad phenotypic spectrum, including reduced cilia number and cell cycle associated cilia morphogenesis. This results ultimately in severe or lethal embryonic malformations and especially osteochondrodysplasia, shortened ribs and tibias, polysyndactyly, fused kidneys, heart defects, mouth cleft, among others[17].

 In terms of molecular functions, a first break through came from a protein interactome study that shedded light on the involvement of Nek1 in several pathways related to the above diseases but also opened new avenues in the context of cellcycle regulation and DNA damage responses[2].Thesefindings were later not only confirmed by functional studies but were also extended to other Nek family members, including Nek4, 6, 10 and 11[3,8,9,18]. The interactome study was a yeast two-hybrid assay using Nek1 as a bait and a human fetal brain cDNA library as a prey. Nek1 is a rather large, 1258 amino acids containing protein and interacts with these proteins mainly through the two N-terminal of its four coiledcoil regions, which are located C-terminally of its kinase domain (Figure 1). Among the Nek1 interacting proteins were the Kinesin-like protein KIF3A, Tuberin and Alpha-catulin, mutation in all three of these genes also have been reported to cause PKD. This suggests the existence of a multi component signaling or regulatory pathway, which regulates the kidney cell’s proliferation and when affected by mutations may lead to PKD[19-21]. Evidence in support for a major role of Nek1 in primary ciliary function came also from other modelorganisms, including *Chlamydomonas*[22].

 Surprising at that time was the discovery of interactions with several cell cycle regulatory proteins – 14-3-3 protein *eta*, YWHAH), Tumor suppressor p53-binding protein 1 (TP53BP1), Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit alpha/delta isoform (PPP2R5A/D) – and especially with proteins involved in the DNA damage response, such as theDouble-strand break repair protein MRE11A (MRE11A) and theTranscriptional regulator ATRX (ATRX)[2]. Soon additional experiments with the irradiation of wild-type and Nek1-/- cells revealed that Nek1 is overexpressed and activated in response to ionizing radiation (IR) and co-localizes to -H2AX positive DNA repair foci in the nucleus[23]. Cells without Nek1 died in response to sub-lethal doses of IR and knockdown of Nek1 also diminished their capacity to clear DNA damage caused by chemical genotoxic agents such as cisplatin and methyl-metanesulfonate (MMS)[24]. This line of experiments culminated recently in a paper where the authors showed that Nek1 kinase is not only physically associated with ATR-ATRIP, but also required for ATR priming to allow an efficient DNA damage signaling[25]. Furthermore, Nek1 has been indicated to act in the apoptosis signaling, especially by phosphorylation of key mitochondrial proteins such as the Voltage-dependent anion-selective channel protein 1(VDAC1)[13]. This is a pore complex that functions both as a voltage dependent anion channel and permeability pore that regulates Cytochrome c leakage to the cytoplasm, which upon exit initiates apoptotic events[13]. Nek1’s activity to maintain cells in homeostasis ismediated through phosphorylation of a specific external VDAC1 Ser residue. Upon apoptotic stimuli Nek1 is degraded and the lack of VDAC1 phosphorylation causes opening of the channel and loss of the membrane potential and leakage of Cytochrome c to the cytoplasm.

 Finally, Nek1 has been implicated in gametogenesis due to its high expression levels in meiotic tissues[26]. In another interactome study, this time using a testicular tissue cDNA library, the protein Nurit was found as an interactor ofNek1[27]. Nurit is expressed in the late phase of spermatogenesis, has structural resemblance with leucin zippers and contains additional super helix domains, possibly involved in its homo-multimerization. Furthermore, the Structural maintenance of chromosomes protein 3 (SMC3) was found to interact with Nek1, further implying important functions in meiotic events such as spindle assembly checkpoints[28].

 In summary, Nek1 has been functionally implied in three major functional contexts and their sub-functions: Ciliogenesis (PKD, SRPS), DNA damage response in a wider sense, also including cell cycle checkpoints and centrosome functions and, finally, gametogenesis. Unpublished recent mass spectrometry studies of the Nek1 interactome after challenging cells with genotoxic drugs identified a number of nuclear proteins, the majority of which associated to DNA repair, replication and transcription regulation. This, together with a very recent article, which reports on Nek1 interaction with NHEJ (Non homologous end joining) repair protein Ku80, clearly establish Nek1 as a key player in DDR signaling[29].

**NEK2**

Nek2 is the most studied and most well understood of the human Neks. In fact it will be difficult to cover all of its aspects in the context of this review. Therefore, we focused on the most important features of Nek2 and would like to apologize to the many researchers whose work could not be covered here due to space restrictions.

 Nek2 shares in its kinase domain the highest sequence similarity with NIMA and many biochemical, structural and functional features. This has led may researchers to believe that it may be the prototype NIMA among all vertebrate Neks and that Nek2 may maintain the primordial functions of NIMA in mitosis progression. For this reason, Nek2 became the most studied Nek family member in mammals[6]. However, care must be taken with such an interpretation, since Nek2 cannot rescue NIMA defective mutants and Nek1 also shares many NIMAcharacteristics[30].

 Nek2 expression varies during the cell cycle, being maximal between the S and G2 phase, during which it localizes predominantly to the centrosome[31, 32]. Nek2 is a component of the MTOC (Microtubule organization centre) at mitosis entry and a core component of the centrosome, where it phosphorylates the centrosomal key components C-Nap1 and Rootletin, which form the intercentriolar linker that holds the pair of centrioles physically together. This event in turn promotes centrosome separation itself[33,34]. During the interphase, Nek2 is maintained in an inactive state by association with the protein kinase MST-2 and the phosphatase PP1, which keeps Nek2 desphosphorylated. After mitosis onset, Polo-like kinase 1(PLK1) phosphorylates MST-2, disrupting the trimeric complex and resulting in Nek2´s activation through auto-phosphorylation. In addition, the centrosomal proteins Nlp (Ninein-like protein) and Centrobin contain coiledcoils and are dislocated from the centrosomes in Nek2 overexpression conditions. In contrast, the Nek2 knockdown or inhibition of its catalytic activity result in the inhibition of the centrosome separation[35].

 A second important functional context for Nek2 is at the spindle assembly checkpoint, wherethrough its interaction with the major kinetochore proteins Mad1/2 and the phosphorylation of the kinetochore core protein Hec1, Nek2 maybe involved in the identification of unaligned sister chromatids [36]. Failure at this checkpoint may lead to aneuploidy and other chromosomal abnormalities andknockdown or knockout of other Neks, including Nek7, has been reported to cause aneuploidy, pointing to a potential major involvement of the Nek family in the spindle assembly checkpoint[37].

 Another functional context for Nek2 is in the gametogenesis, where Nek2 acts in chromatin condensation reminiscent of the role of NIMA in *Aspergillus nidulans*. In spermatocytes, the architectural chromatin protein Hmga2 is under control through phosphorylation by Mitogen-activated protein kinase(MAPK) and possibly also by Nek2[38].

 Finally, in *Drosophila*, Nek2 has been detected at the midbody in the late mitosis and overexpression of Nek2 led to Actin and Actin-binding proteindislocation and cytokinesis failure, among other phenotypic effects[39].

**NEK3**

Nek3 is a 506 amino acid serine/threonine kinase[40] and localizes both to the nucleus and cytoplasm[41,42]. It is highly expressed in testis, prostate, ovary and brain, and shows moderate to low expression in lung and liver[40]. Its gene localizes to chromosome 13q14.2 and its mRNA is expressed in tumor, normal prostate and blood control cell lines. Insertion/deletion polymorphisms were described, in which a stretch of adenines at the end of exon 9, leads to the introduction of a premature stop codon, resulting in a truncated protein that encodes only 298 or 299 of the proteins amino acids. Interestingly, this polymorphism around 13q14 is a mutational hotspot for several cancer types[43-45]. Moreover, Nek3 has an N-terminal catalytic domain and a C-terminal regulatory domain and shares high amino acid sequence identities with mouse Nek3 (56%), but not with other NIMA-related kinases due to the absence of coiledcoil regions (Figure 1)[46].This suggests that Nek3 and its orthologs constitute a separated sub-family of the Neks[40].

Nek3 is involved in the invasion and motility of T47D cells (a human ductal breast epithelial tumor cell line) through interaction with the Guanine nucleotide exchange factor VAV2, which promotes both p21-Rac1 andtransforming protein RhoA activation. These interactions are mediated by prolactin-induced association of Nek3 to the human Prolactin receptor(PRLR). The signaling pathway resulting from prolactin’s binding to its receptor, promotes phosphorylation of Paxillin, a cell adhesion mediator, and is dependent on Nek3’s association with VAV2[41,42].

 In its C-terminal domain Nek3 contains a PEST motif,which contains Thr475, a residue that gets phosphorylated upon activation. TheThr475 and the PEST domains are phylogenetically conserved, suggesting that they are important for Nek’s regulation[47]. Expression of mutants without theThr475 or the PEST domain cause changes in cellular morphology and polarity of both epithelial and neuronal cells. Thus, Nek3 may also be crucial to the regulation of neuronal microtubules and in disorders which involve axonal degeneration, possibly through modification of its acetylation status[47].

Another functional involvement of Nek3 with cytoskeleton components is mediated through its interaction with the EH-domain-containing protein 2 (EHD2). EHD2interacts with plasma membrane phospholipids, associates withVAV1, and forms the complex VAV1-NEK3-EHD2, which modulates p21-Rac1 activity, causing actin reorganization close to the plasma membrane at the initial stages of endocytosis[48]. In summary, Nek3 plays a role in cytoskeleton organization and dynamics, through actin re-organization and may be involved in the regulation of neuronal development, endocytosis, cell motility and invasiveness of breast cancer tumor cells.

**NEK4**

Nek4 was initially described as Serine/threonine-protein kinase 2(STK2) by Cance and co-workers[49]. In a study of a kinase specific cDNA library in human breast cancer tumors or cell lines they identified STK2, that showed homology to *Aspergillus nidulans* NIMA and which expression levels varied widely in human breast tumors. Later, Levedakou and co-workers[50] showed that STK2 is highly expressed in the heart and that its mRNA level does not vary along the cell cycle. After studies characterizing the murine STK2 the nomenclature changed to Nek4[51,52].

 The human Nek4 gene is located on chromosome 3p21.1 and is transcribed into a about 4kb mRNA, which encodes an 841 amino acid residues protein[50]. It is constituted by a N-terminal kinase domain and a C-terminal regulatory domain (Figure 1). Hayashi and co-workers (1999)[51] described a short and a long isoform for murine Nek4. The long mNek4 isoform differs from hNek4 due to the absence of a small fragment in the regulatory domain that corresponds to an *Alu*sequence[51,52]. To date three isoforms have been described for human Nek4. The longest canonical sequence (isoform 1: UNIPROT ID P51957-1, NCBI ID NM\_003157) was identified by the Cance and Levedakou groups[49,50] and used to compare it to mNek4. The isoform 2, only found in the UNIPROT databank (UNIPROT ID P51957-2), is identical to mNek4 and lacks the *Alu* sequence. The isoform 3 (UNIPROT ID P51957-3 and NCBI ID NM\_001193533) is the shortest one, with a smalleralternative N-terminal region.

 Hayashiand co-workers (1999)[51]showed that two isoforms of mNek4 are expressed in most of the tissues, except in the liver and heart where only a short isoform is expressed[50]. Recently, hNek4 expression was also observed in ciliated tissues, such as the retina, kidney tubules, brain (specifically the ventricles), heart and testis[53]. Expression in testis suggests a role in meiosis, as has been already reported for mNek4[52]. Furthermore, these new functional studies demonstrated that hNek4 depletion does not alter the cell cycle[53,54]. Therefore, as shown for other Nek family members, roles other than the regulation of the cell cycle can be attributed to Nek4, including microtubule stabilization, primary cilium assembly and,more recently, replicative senescence entry and DNA damage response[9,53,54].

Interestingly, Nek4 activity is evidenced mainly in the presence of chemotherapeutic agents. For example, in lymphoma cells, a simple Nek4 knockdown is not enough to change cell cycle or microtubule dynamics, but Nek4 knockdown triggers taxol resistance and promotes sensibility to vincristine in these cells[54]. These results indicate thatNek4 has an effect on microtubule stability in the presence of these drugs and suggests that this particularity could be explored in therapies, depending on the patients specific levels of Nek4 protein in the tumor cells.

Besides the direct role in microtubule polymerization, Nek4 is also important for primary cilium stabilization, as was already described for Nek1 and Nek8[14,55,56]. Nek4 interacts with RPGR-interacting protein 1(RPGRIP1) and RPGRIP1-like protein(RPGRIP1L)[53], both associated with ciliopathies. Both, the eye-restricted disease “Leber Congenital Amaurosis” and the “Joubert and Meckel syndrome”, which affects multiple organs, are at the severe end of the ciliopathy spectrum. After Nek4 knockdown the number of ciliated cells decreases, but this effect is apparently not related to RPGRIP1 and RPGRIP1L phosphorylation status. This suggests that Nek4 may act rather as a scaffold for other cilia signaling proteins[53] and,together with Nek1 and Nek8, may be important to other ciliophaties such as PKD[14,55,56].

More recently, the role of Nek4 was also connected to the DDR, because Nek4 depleted cells were found to be resistant to DNA damaging agents, such as etoposide or bleomycin and to γ-irradiation. Besides, Nek4 interacted with DNA-PKcs, Ku70 and Ku80, proteins that have important roles in the NHEJ (Non homology end joining) repair pathway. Nek4 depleted cells also show a decrease of Histone-H2AX activation, probably as a result of an impairment of the DNA-PKcs recruitment[9].

**NEK5**

Among all members of the Nek family, Nek5 is the kinase with the least amount of information. Although identified in different organisms such as *Homo sapiens, Mus musculus, Arabidopsis thaliana*, among others, there is little information about its function and localization. In humans, Nek5 is a protein of 708 amino acids, which kinase domain is located at its N-terminus[4,8]. According to Moniz *et al*[11], Nek5 is the only member of the Nek family that has a Dead Box domain (Figure 1). This domain is involved in cellular processes such as pre-mRNA processing, rearrangement of ribonucleoprotein (RNP) complexes and gene expression[57]. In *Arabidopsis thaliana*, during epidermal cell expansion, Nek5 interacts with Nek4 and 6 and these interactions are important to regulate microtubule organization, probably through the phosphorylation of beta-tubulins[58].Therefore, Nek5 may be associated to the already established cascade consisting of Nek9, 6 and 7 (see details below). However, care must be taken, because the evolutionary gap between mammals and flower-plants is too large to deduce direct conclusions and the functional information on Neks in plants is even scarcer than in mammals[59]. In human cells Nek5 is able to interact with Caspase-3 and this interaction is important for skeletal muscle differentiation[60]. Caspase-3 is a protease involved in mechanisms such as apoptosis and cell differentiation. It was proposed by Larsen *et al*[61] that Caspase-3 activates Caspase-activated DNase to promote and regulate DNA strand breaks introduced into promoter regions of genes encoding effector proteins such as p21 and that this process may represent a more general mechanism of genome alterations that occur during cell differentiation. Since Nek5 is a substrate of Caspase-3 during cell differentiation, other members of this kinasefamily may also be involved in differentiation associated molecular events and this possibility should be explored in future experiments.

**NEK6**

Unlike the other Neks, Nek6 and Nek7 are the smallest and structurally the simplest Neks, consisting only of the catalytic domain with a relatively short N-terminal extension[8]. Although they share significant similarity with each other, being about 86% identical within their catalytic domains, their N-terminal extensions are not conserved, and it has been suggested that they may play a role in the differential regulation of these kinases[3,62]. SAXS experiments, together with SEC-MALS and comparative molecular modeling performed by our group revealed that hNek6 is a monomeric kinase, slightly elongated, with a flexible and disordered N-terminal domain[63].

 Nek6 was initially identified in a classic biochemical screen for kinases capable of phosphorylating the hydrophobic regulatory site of the p70 ribosomal S6 kinase (S6K). Nek6 phosphorylated the Thr412 residue of S6K and other sites, *in vitro* and *in vivo*, suggesting to be a possible regulator of this kinase[64]. Subsequently, though, it was described that Nek6 did not seem to be responsible for the physiological phosphorylation of S6K, SGK or PKB, since it was characterized as having a high preference for a Leu three residues N-terminal to the phosphorylation site of the substrate[65], and more recent evidence supports a NIMA-like mitotic role for Nek6.

 Both Nek6 and Nek7 co-purify with Nek9 as a result of specific interactions and strong binding to a region located between the RCC1 domain and coiledcoil motif of Nek9[66] (Figure 1). The endogenous Nek6 is activated during mitosis, concomitant with an increase in its level of expression, but this requires phosphorylation at the Ser206 residue, which is mediated through Nek9. Nek7 too, is phosphorylated by Nek9 at Ser195, and both phosphorylation sites are found in the activation loops of these kinases[67]. These information led to the construction of a model in which Neks 6, 7 and 9 act as partners of the same signaling cascade[67], with Nek6/7 being substrates of Nek9. However, Nek9 remains inactive during the interphase but is activated during mitosis, phosphorylating and activating Nek6/7, which, in turn, coordinate the organization and maintenance of the mitotic spindle[66].

 Overexpression of a catalytically inactive mutant of Nek6 generates cells displaying high mitotic index, defects in mitotic spindle, nuclear abnormalities and apoptosis[11]. These phenotypes are also observed from the depletion of Nek6/7 in HeLa cells using siRNA, which causes retention of cells in metaphase, with a normal chromatin condensation and alignment, but an inability to complete the segregation of chromosomes. The activity of Nek6 and also 7, therefore, seems necessary for the progression of anaphase, where the cells are either retained at the spindle assembly checkpoint (SAC), or undergo apoptosis, or complete mitosis but with an elevated risk of acquiring chromossomal abnormalities during the process[11,12]. Moreover, treatment of these depleted cells with an Aurora B inhibitor to bypass the SAC led to a reduction in the frequency of metaphase arrest, concomitant with an increase in the frequency of cells blocked in cytokinesis. Cells expressing the hypoactive mutants, even in the absence of the SAC inhibitor, also accumulated in cytokinesis. Therefore, Nek6 and Nek7 seem to have independent, non-redundant roles in mitotic spindle formation and cytokinesis: one at metaphase that requires a certain level of kinase activity and one in late mitosis that requires a higher level of activity[12].

 Intriguingly, using phosphospecific antibodies that detect activated Nek6, Rapley and co-workers[68] showed that Nek6 activity increased 2h after release from a nocodazole arrest, when cells would be progressing through cytokinesis. In this same study, theKinesin-related motor protein Eg5, required for spindle bipolarity, has also been described as a substrate of Nek6. It phosphoryates Eg5 kinesin *in vitro* at several residues, including, Ser1033, which is also phosphorylated *in vivo,* during mitosis, at the spindle poles[68]. A signaling cascade seems to occur where Nek2 first phosphorylates proteins at the intercentrosomal linker in G2 phase, resulting in their dissociation, followed by activation of Nek9 by the Cyclin-dependent kinase 1(CDK1) and the Polo-like kinase 1 (PLK1) in early mitosis and subsequent activation of Nek6 and Nek7. These kinases, in turn, phosphorylate Eg5 (previously phosphorylated by CDK1), promoting the separation of the centrosomes by the motor activity of Eg5, accumulated in the centrosomes[69,70].

 Apart from roles in mitosis, human Nek6 was recently reported by our group to have a broad set of protein partners involved in diverse biological processes[3]. The hNek6 interactome showed that it is a high confidence hub kinase possibly involved in several known and novel cellular pathways, through interactions with and phosphorylation of diverse proteins.Figure 3 depicts some of the main cellular pathways identified for hNek6 based on the interacting proteins retrieved by our screenings. The novel putative pathways shown are the non-canonical Wnt signaling, the Notch signaling and the actin cytoskeleton regulation, whereas the other pathways were already suggested by other studies: the nuclear factor kappa B (NF-κB) signaling[71] and the DNA damage response[18]. In regard to the DNA damage response category identified in our work, many studies show its importance among the tasks triggered by Neks[2, 8-10,18,23-25,72-74].

 On the other hand, Nek6 phosphorylates the transcription factor Oct-1 (POU2F1), a potent regulator of metabolism and tumorigenicity, at S335 in the DNA binding domain, during mitosis, causing Oct-1 to dissociate from the chromatin and concentrate in the centrosomes, spindle poles, kinetochores and midbody[75]. Furthermore, Nek6 phosphorylates histones H1 and H3 *in vitro*, possibly contributing to mitotic chromatin condensation[76]. Nek6 finally also binds the BTB/POZ domain-containing protein KCTD5, which appears to have a role in cytokinesis[77] and apoptosis[78]..

 As the other human Neks, hNek6 was recently found to be linked to carcinogenesis. It shows an increased expression and activity in gastric cancer according to the progression of the disease[79], and up-regulation of Nek6 mRNA correlates with the Peptidyl-prolyl cis-trans isomerasePin1 up-regulation in 70% of hepatic cell carcinomas[80].The overexpression of a catalytically inactive Nek6 promotes cell cycle arrest in human breast cancer in metaphase and leads to apoptosis[11], while its knockdown induces senescence and also apoptosis[81]. In a large-scale screening of serine/threonine kinases on different types of human tumors, Nek6 was shown to be up-regulated in non-Hodgkin’s lymphoma, breast, colorectal, and lung tumors[82]. Moreover, NEK6´s gene, besides AURKA, has its expression increased in esophagitis and esophageal adenocarcinoma, representing a promising candidate marker of these diseases[83]. Recently, it was demonstrated that transcript, protein, and kinase activity levels of Nek6 were highly elevated in the malignant tumors and human cancer cell lines compared with normal tissue and fibroblast cells, indicating an important role for Nek6 in tumorigenesis[84]. Its phosphorylation at Thr210 and Ser206 is critical for the phosphorylation of STAT3 (Signal transducer and activator of transcription 3) at Ser727[85]. Furthermore, its overexpression suppresses p53-induced senescence in cancer cells: it inhibits the cell cycle arrest at both G1 and G2/M transition, the reduction in the Cdc2 and cyclin B levels and the increase in ROS levels induced by p53[86]. Its overexpression also makes cancer cells resistant to premature senescence induced by the anti-cancer drugs camptothecin and doxorubicin[87]. The inhibition of the Nek6 function sensitizes human tumor cells to premature senescence after anti-cancer drug treatment or serum depletion[81], suggesting Nek6 to be a potential therapeutic target for various types of human cancers.

**NEK7**

Human Nek7 was originally described as a possible regulator of the p70 ribosomal S6 kinase[64] and of important events in the mitotic progression[12,6,67,88] (see above for Nek6). These findings have led to studies on the regulatory effects of hNek7 in key functions of the cell cycle and in cancer. The siRNA-mediated down-regulation of hNek7 and expression of kinase inactive mutants reduced centrosomal γ-tubulin levels in interphase cells and caused prometaphase arrest with defects in mitotic spindles[6,87,88]. Nek7 overexpression in culture cells, on the other hand, resulted in multinucleated cells and a higher proportion of apoptotic cells[89]. In the same line, the Nek7 depletion also decreased microtubule stability, while its ectopic overexpression rescued this phenotype[90]. Furthermore, hNek7 deficient mice die early in development and, on a cellular level, lack of Nek7 led to decreased chromosome numbers, increased centrosome numbers, binucleation, micronuclei formation, cytokinesis failure, growth retardation or cell death[37]. The PCM (centrosomal pericentriolar material) proteins do not accumulate at the centrosome inNek7-depleted cells in the G1/S and G2/M transitions[91], indicating that Nek7is required for centriole duplication, centrosome maturation and mitotic spindle formation[88].

 The direct interaction of Nek7 with the non-catalytic domain of Nek9 allosterically activates Nek7 by interruption of its auto-inhibitory conformation[92]. Consistent with these findings, recent studies demonstrated that PLK1 and CDK1 control the centrosome separation through phosphorylation and activation of Nek9 during mitosis. This leads to the Nek6/7-dependent phosphorylation of kinesin Eg5, a key event for centrosome separation and mitosis[69]. Thus, as in the case of Nek6, it is not surprising that cancer cells express elevated levels of Nek7, suggesting a role in tumorprogression. Higher expression levels of Nek7 were found in larynx, breast, colorectal[82] and gall bladder cancers[93]. Taken together, these findings suggest Nek7 as a potentially important regulator of the cell cycle and reveal it as an essential component for growth and survival of mammalian cells. Furthermore, the linkage with a failure in centrosome biogenesis, chromosomal stability and ploidy as well as the observed disturbance of microtubule dynamics connect Nek7 to hallmark features of oncogenesis.

**NEK8**

Nek8 was first described as the mutated gene in murine autosomal recessive juvenile cystic kidney (*jck*) mice[55]. As observed for Nek1, these mutational changes found in Nek8 C-terminal domain can cause genetic kidney diseases, including thepolycystic kidney disease (PKD)[55]. PKD is one of the most frequent genetic kidney diseases and has a highly variable pathology, involving aberrant cell proliferation in the kidney and pleiotropic effects in multiple other organ systems, including the liver and the pancreas. Evidence that renal cyst formation is caused by defects in ciliogenesis or ciliary function is substantial[56]. In mouse cells, Nek8 localizes to the proximal region of the primary cilium and is not observed in dividing cells[56]. In humans, Nek8 is overexpressed in primary breast tumors[94] and localizes to centrosomes and the proximal region of cilia in dividing and ciliated cells, respectively. The localization of Nek8 to centrosomes and cilia is dependent on both the kinase activity and the C-terminal non-catalytic domain homologous to RCC (Regulator of Chromosome Condensation) 1. It is capable of auto-phosphorylation in the non-catalytic C-terminal region to regulate it´s localization or activation. Its activity is not cell cycle regulated, but in the same way as observed for Nek3, activity levels are higher in G0-arrested cells. The kinase domain alone, although catalytically active,does not localize correctly, while a fragment containing only the RCC1 domain shows correct localization and can also be phosphorylated by Nek8[95].

 Nek8 carries the causal mutations of two of the eight established mouse models of polycystic kidneys (*jck*). In these models, an abnormal interaction between Nek8 and the Polycystin complex may give rise to PKD by disturbing microtubule dynamics, the mitotic spindle checkpoint, and the cytoskeleton. Nek8 mutations cause overexpression of Galectin-1, Sorcin, and Vimentin andaccumulation of the MUP (Major Urinary Protein) in renal cysts of *jck* mice[96].

The role of the RCC1 domain in Nek8 is yet unknown. However, a single G448V substitution is responsible for the *jck* phenotype[55]. Overexpression of mutant forms of Nek8 (including G448V) in tissue culture cells leads to the formation of enlarged multinucleated cells and reduced numbers of actin stress fibers, although tubule cells in *jck* mice are not multinucleated, suggesting that the cellular role of Nek8 may be related to the regulation of the cytoskeleton[55].

 Co-immunoprecipitation experiments demonstrated that Nek8 interacts with Polycystin-2 (PKD2), a mecanosensing receptor protein, involved in theregulation of the cilium length. However, the *jck* mutation of Nek8 did apparently not affect this interaction directly. These data suggest that Nek8 interferes with the Polycystins signal transduction pathways and/or the control of the targeting process of these ciliary proteins. Dysfunction of Nek8 may lead to cystogenesis by altering the structure and function of cilia in cells located at the distal nephron[97].

 Recent results suggest that Nek8 has a function in the maintenance of genomic stability[10]. Loss of Nek8 leads to spontaneous DNA damage and a defect in the response of cells to replication stress. Furthermore, Nek8 interacts physically and functionally with components of the ATR-mediated DDR. The disease-related *jck* mutant of Nek8 fails to both interact with the ATR pathway proteins and to rescue the genome maintenance defects associated with Nek8 knockdown. Thus, Nek8 is a critical component of the DDR that links replication stress with primary ciliary functions and the related cystic kidney disorders[98].

**NEK9**

Nek9, also called Nercc1, is one of the largest Neks with 979 amino acids, with an extensive C-terminal regulatory domain, which contains seven RanGEF homology repeats, an RCC1 domain, a segment rich in Ser/Thr/Pro residues and, like in Nek2, a coiledcoil dimerization motif (Figure 1)[66].

 Nek9 was first described as Nek8 and isolated with a catalytic activity against Beta-casein in rabbit lung extracts treated with IL-1, revealing the co-chromatography of a second protein homologous to the Drosophila Bicaudal D protein, Bicd2, which is *in vitro* phosphorylated by Nek9 and resembles a cytoskeleton structure[99]. Moreover, Nek9 immunoprecipitation of *Xenopus laevis* egg extracts showed γ-tubulin and other members of the γ-tubulin ring complex (γ-TuRC), which are essential for the microtubule nucleating activity of the centrosome[98]. Centrosomal γ-tubulin recruitment depends on the adaptor Protein NEDD1 and is controlled by PLK1. In a recent study by Sdelci *et al*[100], it was reported that PLK1 activates Nek9, which phosphorylates the Ser377 in NEDD1, promoting its recruitment together with γ-tubulin to the centrosomes of dividing cells (independent of Nek6/7). Furthermore, the microinjection of anti-Nek9 in human cells during prophase, after the chromosomes condensation, interferes in the organization of the spindles and the proper segregation of chromosomes, resulting in cell cycle arrest in prometaphase or aneuploidy[66].

 Nek9 expression remains constant in different cell cycle phases (G1/S, G2, M, G1), however, as observed for NIMA, there is a specific increase in its catalytic activity during mitosis, which was found to be triggered by *in vitro* and *in vivo* phosphorylation events[66]. The recombinant wild-type Nek9 shows reduced activity when extracted from exponentially growing cells, but its pre-incubation with ATP and Mg2+ induces its autophosphorylation at its activation loop Thr210 residue and its activation, whereas mutants lacking the coiledcoil dimerization motif show significantly reduced activity[66,98]. Interestingly, the deletion of the RCC1 region leads to a catalytic hyperactivity, indicating that this region may be required for Nek9 autoinhibition[66]. Moreover, Nek9 binds to Dynein light chain 1, cytoplasmic(DYNLL1), a highly conserved protein originally described as a component of the dynein complex, via its C-terminal (K/R) XTQT motif adjacent to Nek9 C-terminal coiledcoil motif, resulting in Nek9 oligomerization, an increase in its autoactivation rate and a reduction in its binding to Nek6[101].

 It is possible that Nek9 activation in mitosis involves a very small percentage (< 5%) of the total expressed protein, and in contrast with the vast majority of inactive protein, the active Nek9 (Thr210P) is first evident during prophase, concentrated at the centrosome, where it can be phosphorylated by CDK1/cyclin-B[102], until metaphase is reached. During the transition to anaphase, the immunoreactivity of Nek9 (Thr210P) decreases at the centrosomes and becomes detectable at the chromosomes, which is evident until telophase. Before disappearing, the active Nek9 is detected at the midbody as two points flanking the cleavage furrow during cytokinesis[98].

 Due to its possible roles in the mitotic spindle organization and chromosome segregation through its activation during mitosis and interaction with Nek6/7, it is possible that most of the phenotypes observed with the microinjection of anti-Nek9 antibodies in human cells are caused by interference with Nek6/7 function[66]. Taken together, the data suggest that Nek9 is a positive upstream regulator of Nek6/7.

 Among other kinases, Nek9 was recently identified by quantitative chemical proteomics as a possible marker for the diagnosis andtherapy of head and neck tumors[103]. Moreover, Nek9 shows, along with other kinases implicated in cancer, its activity inhibited by the drug quercetin[104]. Its expression is increased in chronic myeloid leukemia cells resistant to imatinib[105], indicating that its up-regulation could be involved in chemotherapy resistance mecanisms. Depletion of Nek9 in glioblastoma (U1242) and renal carcinoma (Caki2) cells results in failures in cytokinesis, and cell death in Caki2 cells, after overriding mitosis, and incorrect alignment of chromosomes and micronuclei formation. Therefore, it is suggested that inhibition of Nek9 is a potential anti-cancer therapeutic strategy by induction of mitotic catastrophe via reduced dynamics of the spindle, cytokinesis and mitotic checkpoint control[106].

**NEK10**

One of the most intriguingbut to date less studied members of the Nek family is Nek10, since it has its catalytic domain flanked by two regulatory domains (Figure 1). Each of these two regulatory domains have their own peculiarities. As NIMA and Neks 1, 2, 5, 9 and 11, Nek10 has also coiled-coil regions closely located to the kinase domains[8]. Furthermore, four repetitions of an armadillo repeat motif in its N-terminal regulatory domain may serve as an important region for protein-protein interactions, as it has been reported for other proteins[107]. In the case of its C-terminus, a PEST region may be important to the proteolytic regulation of the protein’s abundance. There are some contradictions and a debate about Nek10 full length, since several different cDNAs have been deposited that differ in the C-terminal domain length.

 Mutations in the Nek10 gene locus have been linked to breast cancer in different studies that were trying to find new polymorphisms in carriers of mutations in BRCA1/2 (Breast cancer type 1/2 susceptibility protein)[108-110]. Moniz and collaborators[74] have shown an important role for Nek10, comparing normal and tumor mammary gland cell lines. They found that Nek10 affects the ERK1/2 (Extracellular signal-regulated kinase 1/2) signaling pathway, after activation with UV radiation. Nek10 has been shown to form a functional complex with RAF1 and MEK1 (Dual specificity mitogen-activated protein kinase kinase 1). In this sense cell cycle arrest in G2/M was observed, and Nek10 caused both MEK1 activation and the ERK1/2 phosphorylation. Altogether, these preliminary data suggest a possible involvement of Nek10 in the DDR as already demonstrated for Nek1, 4, 6, 8 and 11[2,8-10,18,23-25,72-73]. Moreover, like BRCA1 and BRCA2, Nek10 may be a therapeutic target in breast cancer.

**NEK11**

Nek11 is one of the least studied Nek family membersand has the highest sequence similarity to Nek4. Its gene is present on the same chromosome as that ofNek4, however on the long arm (3q21-22). Nek11 was first identified by Noguchi and co-workers (2002)[111] and shows a high sequence similarity with Nek4 and 3 in its kinase domain, but is more similar to Nek2 in its regulatory region (Figure 1). Interestingly, Noguchi and co-workershave not found Nek4/11-related kinases in *C. elegans* or *D. melanogaster*, suggesting that the Nek11-containing subfamily may have only appeared through gene or genome duplication after separation of the deuterostome branch in the animal kingdome[111].

Noguchi and co-workers (2002)[111] described two isoforms for Nek11. The longer isoform (Nek11L) is composed by 645 residues, while the shorter one (Nek11S) contains only 470 residues. Nek11 shows a N-terminal kinase domain and a C-terminal regulatory domain with a coiled coil and three PEST sequences, suggesting a proteolytic, cell cycle specific regulation of its expression. Nek11, different from Nek1, 2 and4, is not present in a higher quantity in the testis or ovary, but its mRNA is found in the brain’s cerebellum, trachea, lung, appendix, and uterus[111]. Another important difference to Nek4 is that Nek11 shows a timely cell cycle related expression pattern, relating it closer to Nek2 with both showing an expression peak at the G2/M transition.

The first indication that Nek11 could be important in the regulation of cell cycle checkpoints, was the identification of histones H1, H2A and H3 as Nek11 phosphorylation substrates. Furthermore, inthe presence of genotoxic agents, Nek11 showed both an increased expression and activity at the G2/M transition. Thoughthis is decreased by caffeine, suggesting that Nek11 DDR may be associated with the ATM/ATR pathways, which also showed the same inhibition by caffeine[111].

Another common point between Nek11 and Nek2 is theirlocalization to the nucleolus. In the study of Noguchi and co-workers (2004)[112], it was observed that in U2OS cells Nek11L is present in the nucleolus during interphase and telophase and that it probably interacts with Nek2A in the nucleolus. Moreover, Noguchi and co-workers speculated that Nek2A could phosphorylate Nek11L C-terminal and, in this way, antagonize its auto-inhibitory function, which would cause Nek11 activation in G1/S arrested cells[112].

Recently, some of Noguchi results were followed up by Melixetian and co-workers[73]. This study points to Nek11 as an important player in cancer development. Melixetian and co-workers[73] observed that Nek11 depleted U2OS cells loose an important G2/M checkpoint after IR. In this way it was verified that after IR Chk1 phosphorylates both M-phase inducer phosphatase 1 (CDC25A) and Nek11. Nek11 in turn also phosphorylates CDC25A, leading to its proteasomal degradation and subsequent inhibition of Cyclins followed by a cell cycle arrest at the G2/M transition.

The studies involving Nek11 so far point to it as an important protein for the cell cycle regulation in the context of the DDR. However, more interactome studies are required to clarify other possible functions of Nek11 in the cell.

**DISCUSSION**

After knowing sufficient details on all of the eleven individual Neks, we will now return to a more general and integrative approach and try to find common functional contexts for the family as a whole in human cells. As pointed out in the introduction, Neks may be assigned to three major functional contexts: (1) centrioles and mitotic spindle functions; (2) primary ciliary function; and (3) G2/M phase associated DDR. Although most individual Neks have been associated to one main context, recent functional data as well as the identification of interaction partners for several Neks from two or even all three contexts may suggest that Neks have a broader function, possibly on a regulatory level, that consequently affect the three main functions. A first way of looking at this is by comparing the interaction profiles and functional contexts of the published interacting partners, summarized in Figure 2, which shows the Neks global interaction profile and the possible new biological processes in which they are involved due to their interaction with multiple proteins.

Several protein interactors with violet color interact with Nek1, 2, 3, 8, 9 and 11 and can be described as associated to the “axon guidance”/transport processes. They include for example Fasciculation and elongation protein zeta (FEZ)-1 and -2, that interact with Nek1[2,113,114].

Several proteins associated to apoptotic processes interact with Nek6: Serine/threonine-protein kinase PAK 6 (PAK6), Serine/threonine-protein kinase Sgk1 (SGK1) and DBIRD complex subunit KIAA1967 (KIAA1967) (darker green color).

Nek9 interacts with several proteins from the Autophagy-related protein 8 family (GABARAP, GABARAPL1, GABARAPL2, MAP1LC3A, MAP1LC3B and MAP1LC3C) (light blue).

Several proteins from DNA repair processes interact with either Nek1,6,9 or 10: RuvB-like 2 (RUVBL2), Fanconi anemia group I protein (FANCI), Transcriptional regulator ATRX (ATRX), FACT complex subunit SSRP1 (SSRP1), and SUMO-1 (SUMO1) (red). The Putative DNA repair and recombination protein RAD26-like (RAD26L), the PHD finger protein 1 (PHF1), and also the Double-strand-break repair protein rad21 homolog (RAD21, not shown in Figure 2), all identified as Nek6 interactors in our yeast two-hybrid screens[3], are also possibly involvedin the DDR[115,116].

In order to demonstrate the potential discovery of additional functional contexts through interactomics studies, we will now have a closer look at the Nek6 interactome as described by our group[3] (Figure 3). Novel Nek6 interacting partners are indicated by yellow ellipses, and suggest the following new functional contexts: (1) Nek6 is possibly involved in actin cytoskeleton organization through its interaction with cell division control protein 42 homolog (CDC42) and Sorting nexin-26 (SNX26)[3]. Since SNX26 has a negative regulatory role on CDC42 and Nek6 interacts with both of them, the final output of Nek6 must be adressed by future experiments. However, these findings are supported by the fact that for Nek3 a clear involvement in related processes has been reported (see Nek3 section above); (2) Nek6 may be involved in the activation of the NF-κB signaling on multiple layers, since it interacts with the Transcription factor RelB, Prx-III and/or TRIP-4[3,71]. Matsuda and co-workers have found Nek6 as an activating protein in an siRNA knockdown screen to identify proteins that participate in the regulation of cellular survival transcription factor NF-κB. The regulation may occur on several levels: through direct phosphorylation, interaction or regulation of the nuclear translocation of key components of the NF-κB complex, like RelB, or even on the transcriptional level. The latter seems likely, since Nek6 also interacts with SNW domain-containing protein 1 (SNW1) and a PHF domain containing protein (PHF1)[3], both of which have been recently identified as key components involved in the complex, multiprotein machinery involved in the transcriptional activation of the NF-κB gene[117]. Again, Nek6 regulatory role here may be mediated through interaction and/or phosphorylation; (3) the IR-induced DNA damage response is mediated by Nek1, 6 and 11, leading to cell cycle arrest[18,23,25,72,73]. The UV-induced DNA damage response is mediated by Nek10, also leading to cell cycle arrest[74]. This may suggest that different Neks may have specialized to mediate different forms of DNA damage responses; and (4) furthermore, it is known that Nek6 can counteract p53 induced senescence[81]. As we can observe in Figure 3 this may occur indirectly through Nek6 modulation of p53 interactors 40S ribosomal protein S7 (RPS7) and/or E3 ubiquitin-protein ligase RBBP6 (RBBP6). It is worth noting here that Nek4 has the opposite effect of Nek6. Nek4 seems to be required for the cell to enter in senescence[9].

Another important point is the finding that certain functions first only described for isolated specific Neks have been later confirmed for most if not all other Neks. Nek1 was the first family member to be associated with DDR signaling events[23]. In our yeast two-hybrid screen to identify Nek1 interacting proteins we identified proteins involved in the repair process itself (MRE11A) and in different signaling pathways associated to it (ATRX, PPP2R5 A/D, YWHAH, TP53BP1) (Figure 4).

Nek4, 6, 8, 10 and 11 have also been reported to physically interact with key members of DDR pathways or to interfere functionally in signaling cascades in a broader context of the G2/M transition[8-10,18,73-74]. As described above for Nek6, the interactors RAD26L, PHF1, RAD21[3], FANCI and RUVBL2[123] are all associated to the DDR. Together with the relatively recent work byLee and co-workers (2008)[18], this suggests Nek6 may also interfere in DDR. However, the stimuli that activate such possible pathways via Nek6 are still unknown. In further yeast two-hybrid screens and mass spectrometry interactomics studies we found other DDR members interacting with Nek3, 4, 5, 7, 8, and 10 (unpublished data). Recent publications clearly confirmed part of those findings or went beyond them by characterizing this new involvement not only functionally, but also establishingpossible cross-connections between primary cilia signaling and DDR in the case of Nek8[10]. For Nek4, an involvement in senescence signaling was established and in mass spectrometry experiments several DDR proteins such as DNA-PKcs (PRKDC), Ku70/Ku80 (XRCC6/5) and PCNA were identified as Nek4 interacting proteins (Figure 4)[9]. Furthermore, Nek4 has been reported to interact with RPGRIP at the primary cilium[53], thereby establishing another link between DDR and primary cilium function.

A new role for Nek5 in differentiation and apoptosis signaling has been identified and characterized through its interaction with andproteolytic processing by Caspase-3[60]. Evidently, apoptosis signaling is closely related to DDR and the G2/M checkpoint, because cells unable to repair major DNA damage must either halt in the cycle or be dispatched by apoptosis. The link between Neks, DDR and apoptosis is not new indeed, since Chen and co-workers had also already reported an interaction of Nek1 with mitochondrial VDAC1[13]. Nek1 phosphorylates VDAC1 and prevents apoptosis by avoiding VDAC1 opening and leakage of Cytochrome c, which would activate apoptotic caspases. The down-regulation of Nek1 protein level or kinase activity through apoptosis signaling decreases VDAC1 phosphorylation, results in its opening and leakage of Cytochrome c, thereby activating the apoptosis program.

For Nek1, the coexistence of functional roles in both DDR[118] and ciliopathies and primary cilia function had been long established (Figure 4). Nek1 interacts with several proteins involved in the primary cilia function and especially in kidney duct mechanosensing (KIF3A, Tuberin, Alpha-catulin, Polycystin 1/2). Mutations in the genes that encode all of these proteins like those that cause expression of truncated non-functional Nek1 itself, cause PKD[14]. Since Nek8 is functionally and evolutionary most closely related to Nek1 among the Nek family, it came as no surprise that Nek8 mutations were also found to cause ciliopathies and cystic kidney disease. Moreover, Nek8 interacts with some key DDR proteins, including ATR, Chk1 and PCNA, too, just like Nek1[10]. What is new however, in these milestone discoveries, is the possibility that somehow these two pathways are causative or coincidentally connected. Choi and co-workers made the observation that mice cells with diminished Nek8 kinase activity, simulating a kidney ciliopathy, show already in the embryonic phase a constitutive activation of DDR pathways, as evidenced by repair foci in their kidney cells nuclei. This raises a couple of possibilities to consider: either the cilia have some function in the sensing of DNA damage or in transmitting downstream events, or otherwise, the cilia defects somehow transduce (via Nek8) to a possible lack of repair of replication defects. Of course a simpler explanation could be that both phenomena are affected simply because Nek8 participates in both of them simultaneously. However, an additional possibility is that Nek8 acts on a higher regulatory level that coordinates both pathways based on the necessity of the cell to coordinate these events closely during the course of the cell cycle. Clearly, further studies are necessary to evaluate these new possibilities. However, it seems to be clear now that the three central functions controlled by Neks – mitosis, primary cilia and DDR – are more connected than previously expected and that several if not all Neks participate in more that one of them.

Apossibility exists that the Neks *per se* are the key regulatory elements that may connect these three functions. The seemingly functional redundancy may in fact rather represent connecting elements between hitherto non-connected regulatory circuits (Figure 5), *e.g.,* between primary ciliary function and DDR for Nek8[10] and Nek1[2,23,14]. Furthermore, these circuits may cooperate in a concerted one- or two-directional fashion (Nek8).

Most interestingly, from a cilium perspective, recent evidence is also indicating a strong link between cilia, stress responses and DNA damage repair processes. A recent study showed that environmental stresses, including UV and IR, result in altering the protein composition of centriolar satellites, thereby promoting de novo ciliogenesis[119].Together with the recent findings that ciliopathy-associated mutations in DNA damage key regulators (*e.g.,* Mre, Znf423)also connect cilia and DDR[120-127], it is tempting to speculate that cilia may act as platforms for cell cycle checkpoints or the DDR.

**CONCLUSION**

Clearly, the past 10 years have provided new and exciting insights into the multifaceted functions of this interesting protein kinase family and the future promises to hold more surprises and the discovery of new functional connections. An exciting time has come to the field of Nek research and the Neks are ready to step out of the shade and take a main role along the other important cell cycle regulatory kinases: Polo-like kinases, Aurora kinases, and Cyclin-dependent kinases.It istime to stop Ne(c)king around with them and allow them to enter the spot light in the field of cell cycle biology.

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**Figure1 Representation of the domain organization of the eleven human Neks depicting the domain regions for selected protein interactions**. The gene symbols corresponding to interacting proteins are shown above the Neks primary structure regions with which they have been found to interact. The list of interactors is not intended to be complete but necessarily shorter than the list of all proteins known in the literature to interact with Neks (*e.g.,* see Figure 2), since for the majority of interactors the location of interaction in the Neks has not been reported. Different repeated domains have been indicated by the color codeat the bottom of the figure. The lengths of the full proteins are indicated by number of amino acids (aa) at the C-terminal of the proteins. At least two isoforms of Nek1, 2, 3 and three of Nek4 and 11, all generated by alternative splicing, have been reported and known functional distinctions have been briefly discussed in the text, where feasible. References for the proteins and their mapped interactors: Nek1[2, 13, 25]; Nek2[116,126-129]; Nek4[53]; Nek6[3]; Nek9[66].

**Figure 2 Global interactome of Nek1-11, involving their published interactors.** The proteins color code refers to their main biological function given by the top enriched Gene Ontology[130] biological processes (*P* ≤ 0.05). Common interactors establish crosslinks between Neks, thereby emphasizing their common functional contexts. The protein sizes are depicted proportional to their connectivity degree. The protein-protein interaction network was built for the first neighbors of Neks using the Integrated Interactome System (IIS) platform, developed at National Laboratory of Biosciences, Brazil (<http://www.lge.ibi.unicamp.br/lnbio/IIS/>) and visualized using the Cytoscape software[131].

**Figure 3 Nek6 interactome and the cellular functional contexts based on its interacting proteins.** The four major pathways discussed in the text are: (1) Actin cytoskeleton organization; (2) NF-κB signaling; (3) DNA damage response; (4) p53 signaling. According toMeirelles and co-workers[3]. See detailed legend for symbols at the bottom of the figure. IR: ionizing radiation.

**Figure 4 Nek1 interactome and cross talk with other Neks and protein interactors in the context of the DNA damage response pathways.** Interactions between proteins are depicted as simple lines, activation is depicted as an arrow and inhibition as an arrow with a line as arrowhead. A red arrow for 14-3-3 means that it causes activation by the transport of CDC25 to the nucleus. Nek1 interactedwith a specific 14-3-3 isoform called YWHAH[2] (gene symbols inside brackets correspond to the isoforms of those proteins which were described to interact with Nek1). Not necessarily the same specific 14-3-3 protein promotes the indicated functions. Rather a family characteristic is intended to be assigned. Nek2 kinase activity is inhibited after DNA damage (↓)[132]. The red protein names are those that have been identified to directly interact with Nek1 as identified by the yeast two-hybrid system[2] or other as indicated in the figure. Gene symbols above/under protein names represent other interactors of those proteins. Nek4 interactors have been identified by mass spectrometry[9]. As can be seen, all but three Neks (Nek3, 7 and 9) seem to be directly linked to the DNA damage response. Most strikingly, we can see a direct connection for Nek8, 4 and 1 between DDR and primary cilium function and ciliopathies. New connections to apoptosis have been recently pointed out for Nek1 and 5. References for interactions are depicted in brackets: Nek6[3, 123]; Nek1[2, 13, 25]; Nek4[9, 53]; Nek8[10]; Nek11[73]; Nek10[74]; Nek2[132]; Nek5[60]; KIF3A[19]; Fez1/2[133], various known interactions[134].

**Figure 5 Functional overlap in the human Nek kinase family: seven of eleven Neks participate in two and one Nek in all three of the main core functions of the Nek family (Centrosome-related mitosis, Primary cilia and DNA damage response).** The three corners of the triangle represent each a key concept function for the Nek family, *e.g.,* Nek9 and 11 sole involvement in mitosis[66,67] and DDR[73] respectively, has been well documented. The Nek names and bold lines represent cases where accumulated experimental evidence strongly suggests a regulatory role for that Nek in that context or in both of the contexts the line connects:Nek1[2,22,23]; Nek2[128]; Nek4[9,53] (Basei *et al* unpublished); Nek6[3]; Nek7[67]; Nek8[8,10]; Nek10[74]. The thinner lines represent our own groups preliminary or unpublished interaction data (both from yeast two-hybrid system and immune-precipitation coupled to mass spectrometry analysis data), suggestive of a participation of that Nek in both connected functions (Nek7: Souza et al., unpublished; Nek10, Papa *et al*., unpublished).

**Table 1 Subcellular localization, established and possible additional functions of human and mammalian Neks**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Nek** | **Gene/ protein****synonyms** | **Subcellular localization** | **Established function** | **Possible additional functions** **(under investigation)** |
| 1 | NY-REN-55SRPS2,SRPS2A, KIAA1901 | cytoplasm, cilia, centrosome, H2X positive DNA damage sites in nucleus | - stability and function of the primary cilium/polycystic kidney disease[14]- DNA damage response to IR and chemical mutagens[2,23-25] | - meiosis[26-28]- apoptosis mediated by mitochondria[13] |
| 2 | NEK2A, NLK1, RP67,HsPK21, SRPS2A | centrosome | - regulation and promotion of centrosome segregation[33-35] | - DNA damage response[127] |
| 3 | HSPK36, RP11-248G5.5 | cytoplasm | - regulation of prolactin response[41]- microtubule deacetylation in neurons[47] | ? |
| 4 | STK2, NRK2, pp12301 | cilia/ basal bodies | - microtubule stability (silencing alters sensitivity to vincristine/taxol)[54] | - DNA damage response[9]- replicative senescence[9]- primary cilia function[53] |
| 5 | - | ? | - skeletal muscle differentiation[60]- casapase-3 substrate/ apoptosis[60] | ? |
| 6 | SID6-1512,RP11-101K10.6 | mitotic spindle, centrosome | - mitotic spindle formation[11-12]- centrosome separation[69-70] | - DNA damage response[18]- NF-kappaB signaling?[3,127] |
| 7 | - | spindle poles | - mitotic spindle formation [12,88]-centrosome separation[69-70] | - DNA damage response?1 |
| 8 | JCK, NEK12A,NPHP9, RHPD2 | centrosome, cilia,H2X positive DNA damage sites in nucleus | - stability and function of the primary cilium/polycystic kidney disease[95]- DNA damage response[10] | - integration of primary cilia function and DNA damage response[10] |
| 9 | NERCC, NERCC1,KIAA1995 ,(NEK8) | spindle poles, centrosome, cytoplasm | - mitotic spindle formation[106]- centrosome separation[100] | ? |
| 10 | - | Centrosomal pericentriolar material2 | - DNA damage response after UV induced damage[74] | centrosome function?2 |
| 11 | - | nucleus, nucleoli | - DNA damage response induced by IR[73] | ? |

1 Souza *et al*, unpublished observation; 2Papa *et al*, unpublished observations.