Minireview

NDR family of AGC kinases – essential regulators of the cell cycle and morphogenesis

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Abstract The nuclear Dbf2-related (NDR) family of protein serine/threonine kinases comprises mammalian NDR and large tumor suppressor (LATS) kinases, their orthologs from *Drosophila melanogaster* and *Caenorhabditis elegans*, and a number of related kinases from yeast and plants. The members of this family were independently implicated in various aspects of the control of cell division and morphogenesis. They are crucial regulators of the actin and tubulin cytoskeletal organization during polarized growth and cytokinesis in yeast. Furthermore, they are key players in control of proliferation and morphology of many cell types in *D. melanogaster* and *C. elegans*. In mammalians, the LATS kinase is a tumor suppressor, negatively regulating the cyclin-dependent kinase CDK1, cell proliferation rate, and modulating cell survival.

Key words: NDR; LATS; AGC group; Protein kinase; Morphogenesis; Cell cycle

1. Introduction

The NDR (nuclear Dbf2-related) family of protein serine/threonine kinases (Fig. 1) is a conserved subclass of the AGC (protein kinases A, G, and C) group of kinases, and it includes mammalian protein kinases NDR1 and NDR2, *Drosophila melanogaster* TRC, *Caenorhabditis elegans* SAX-1, mammalian *D. melanogaster*, and *C. elegans* large tumor suppressor (LATS) kinases, *Neurospora crassa* Cot-1, *Ustilago maydis* UKC1, *Saccharomyces cerevisiae* Cbk1, Dbf2 and Dbf20, *Schizosaccharomyces pombe* Orb6 and Sid2, and a number of plant kinases [1]. The members of the NDR family are more distantly related to other conserved protein kinases such as the Ghenghis Khan (GEK) kinases DMPK and MRCK, the ROCK kinase, and the budding yeast kinase Rim15 (sometimes considered also as a NDR family member).

Regarding their kinase domain sequence, NDR family kinases are related to the other members of the AGC group kinases, e.g. PKA, PKB, PKG, PKCs, PRK, $p70^{S6K}$, $p90^{RSK}$, and PDK1. They contain all 12 subdomains of the kinase catalytic domain as described by Hanks and Hunter [2]. However, a unique feature of NDR family kinases is an insert of about 30–60 amino acids located between subdomains VII and VIII. The function of this insert is not well understood, but in the case of NDR1, it accommodates a non-consensus nuclear localization signal [3]. In addition, all members of the NDR family have a conserved but poorly characterized N-terminal regulatory domain of different length. In NDR, this domain consists of 87 amino acids, and it encompasses a region which is predicted to form an amphiphilic α-helix responsible for binding the calcium sensor protein S100B [4]. Finally, the C-terminal extension contains a broadly conserved hydrophobic phosphorylation motif that is an important regulatory element within the AGC group of protein kinases [5].

Apart from their high sequence homology, the function of the NDR family kinases (Table 1) has been conserved over long evolutionary distances, mainly involving various aspects of cell division and morphogenesis. In this article, we review the modes of regulation of NDR family kinases, and summarize the current knowledge about physiological functions of these kinases in different organisms.

2. Regulation of NDR protein kinase family

The molecular mechanisms of regulation of protein kinases from the NDR family are best studied for the human NDR1 isoform which is presumed to serve, due to the high homology in the catalytic and regulatory domains among family members, as the prototype for the regulation of this kinase family. Although the physiological upstream signal for NDR activation was not yet identified, NDR is potently activated in vivo by treatment with the protein phosphatase 2A inhibitor okadaic acid, indicating involvement of phosphorylation at serine/threonine residues in the regulation of NDR activity. Indeed, we demonstrated that activation of NDR1 involves phosphorylation of two regulatory residues, Ser-281 from the activation segment, which is located immediately next to the kinase domain insert, and Thr-444 from the hydrophobic motif located in the C-terminal region (Fig. 2 and [6]). The importance of these residues is not surprising, because similar observations were also made for numerous other AGC group kinases (reviewed in [7]). The regulatory potential of phosphorylation of

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Abbreviations: CaM, calmodulin; CDK, cyclin-dependent kinase; GAP, GTPase-activating protein; GTPase, guanosine 5′-triphosphate-binding protein; MEN, mitotic exit network; SIN, septation initiation network; SMA, S100B and Mob1 association; SPB, spindle pole body

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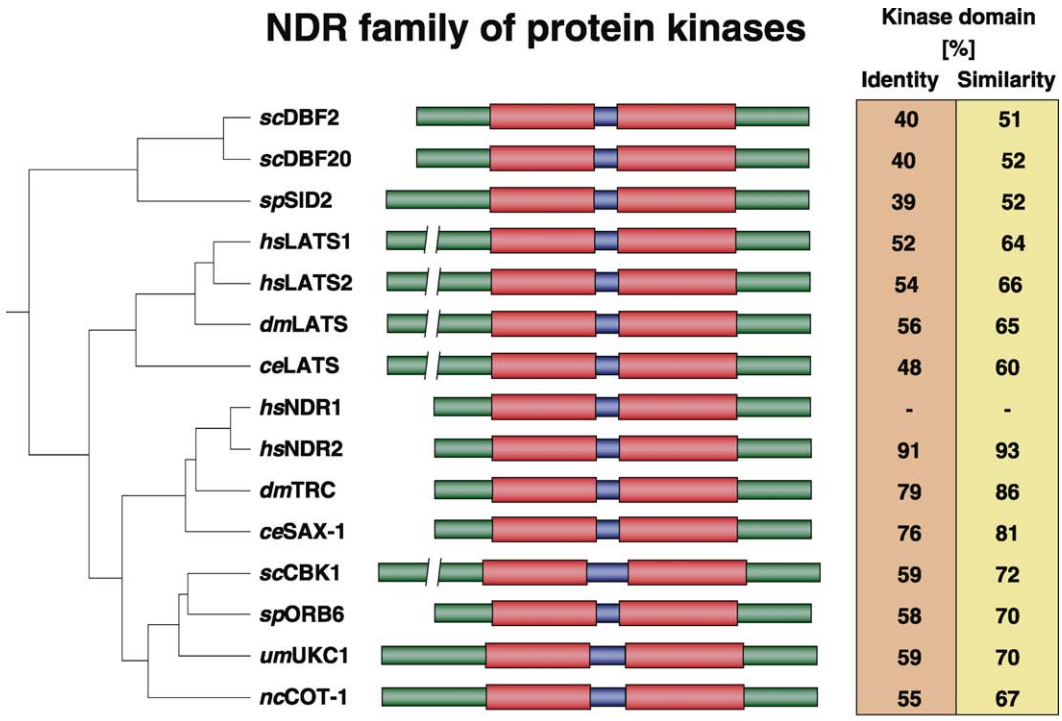

Fig. 1. NDR family of protein kinases. The NDR family of protein serine/threonine kinases is a subclass of AGC kinase group. Its members are highly conserved in evolutionary distant species and are implicated in various aspects of cell morphogenesis and growth control. The phylogenetic tree illustrates the relationship among the full-length protein sequences of the different NDR family members from *Homo sapiens* (*hs*), *D. melanogaster* (*dm*), *C. elegans* (*ce*), *U. maydis* (*um*), *N. crassa* (*nc*), *S. cerevisiae* (*sc*), and *S. pombe* (*sp*) (constructed using GrowTree sequence analysis program from Wisconsin Package software). Schematic diagrams of the NDR family kinases depict the kinase catalytic domains (red), kinase domain inserts (blue), and the N- and C-terminal extensions (green). On the right, the percentage of sequence identity and similarity between the kinase catalytic domains of NDR family members and the catalytic domain of human NDR1 is indicated.

the budding yeast NDR homologue Dbf2 at two equivalent residues Ser-374 and Thr-544 supports this mechanism [8]. Based upon structural analysis of the prototypical AGC kinase PKA, the activation segment residue is found in its phosphorylated form to align the catalytic site of this enzyme, thereby generating an active kinase conformation [9]. The recently resolved structure of PKBβ confirmed the crucial role of the activation segment residue, and defined the function of the hydrophobic motif site which, upon phosphorylation, undergoes a series of interactions with αB- and αC-helices of the catalytic domain, thereby promoting disorder to order transition of this part of molecule with concomitant restructuring of the activation segment and reconfiguration of the kinase bilobal structure [5,10].

In our recent work, we found that the Ser-281 site is an autophosphorylation residue in vivo because kinase-inactive NDR1 mutant does not become phosphorylated at this position. Hence, the kinase activity of NDR1 is required for the phosphorylation on Ser-281 in the activation segment [11]. In most other AGC kinases including PKB, $p70^{S6K}$, SGK, $p90^{RSK}$, and PKC isoforms, however, the activation segment residue has been reported to be a target of phosphoinositide-dependent kinase PDK1 (reviewed in [12]). The pivotal role of PDK1 in phosphorylation of AGC kinases is supported by the high conservation of activation segment residues within the AGC family and by the fact that a number of AGC kinases can directly interact with PDK1. However, more recent studies employing $PDK1^{-/-}$ ES cells confirmed that there exist several exceptions to this general concept. Some AGC kinases such as PKA, PKCδ, AMPK, MSK1, and PRK2 become efficiently phosphorylated at their activation segment residues in absence of PDK1 [13,14], and at least two of these kinases undergo autophosphorylation [15,16]. Our earlier observations on NDR1 demonstrated that this kinase is also not modified by PDK1, neither in vivo nor in vitro [6]. This can be explained by the fact that the NDR1 sequence surrrounding Ser-281 (see Fig. 2) displays obvious variations in P+1 and +2 positions to the highly conserved PDK1 consensus target site (*Ser*/*Thr*-Phe-Cys-Gly-Thr-Xaa-Asp/Glu-Tyr-Xaa-Ala-Pro-Glu, where *Ser*/*Thr* is the phosphoacceptor site and Xaa stands for a hydrophobic residue). Thus, in contrast to most AGC group kinases, NDR is not targeted by PDK1, but instead it becomes efficiently autophosphorylated at the activation segment residue both in vivo and in vitro.

In contrast to the activation segment residue, the hydrophobic motif site Thr-444 is targeted by an as yet unidentified upstream kinase, because the kinase-inactive NDR1, as well as the Ser-281 alanine mutant, become phosphorylated at Thr-444 virtually to the same extent as wild-type NDR1 in vivo [11]. Significantly, Thr-444 is flanked by hydrophobic amino acids which are in this region conserved among almost all AGC group kinases (consensus Phe-Xaa-Xaa-Phe-*Ser*/*Thr*-Phe/Tyr; except NDR1 has a tyrosine instead of phenylalanine at the P−1; see Fig. 2). There is an ongoing dispute concerning the mechanism of action and identity of upstream kinases phosphorylating the hydrophobic motif residues in AGC protein kinases. For the NDR kinase family, there are some significant clues suggesting that the upstream kinases are members of the Ste20 family. First, the upstream kinase of Dbf2 was identified as Cdc15, one of the budding yeast Ste20-

like kinases [8]. Second, the fission yeast Ste20-like kinase Pak1/Shk1 was reported to genetically interact with NDR homologue Orb6 [17]. Third, two other Ste20 family kinases, Cdc7 and Sid1, were placed upstream of the second fission yeast NDR homologue Sid2 [18].

Apart from the phosphorylation-mediated control of NDR kinase activity, NDR family kinases appear to be regulated also through their conserved, N-terminally extended regulatory domains. In NDR1, this domain consists of 87 amino acids and it encompasses a region which is predicted to form an amphiphilic α-helix responsible for the binding to S100B, an EF-hand Ca^{2+}-binding protein of S100 family [4]. We found that Ca^{2+}/S100B promotes, in a Ca^{2+}- and concentration-dependent manner, both autophoshorylation of NDR and its transphosphorylation activity towards an exogenous peptide substrate. Furthermore, Ca^{2+}/S100B increases NDR1 phosphorylation on the two regulatory sites Ser-281 and Thr-444 [11]. Recently, a class of broadly conserved proteins called Mob (from Mps one binder [19]), which display no significant homology to other known polypeptides, was identified to bind and activate several members of the NDR family (see Section 3). Our unpublished observations suggest that these proteins might bind to NDR kinase also through the N-terminal regulatory domain, in a manner similar to the binding of S100B. Therefore, we term this NDR regulatory region the SMA (S100B and Mob1 association) domain. In the classical calmodulin (CaM)-dependent kinases (and also in the twitchin kinase), the autoinhibitory CaM/S100-binding domain is located directly C-terminal to the kinase catalytic domain, and sterically hinders the access of substrates to the active site of kinase [20]. This inhibition is relieved upon binding of CaM or S100 proteins. The mode of action of the SMA domain, however, seems to be different. It is located N-terminal to the kinase domain, and it does not appear to be autoinhibitory since its deletion results in a kinase with significantly attenuated activity [4]. Thus, S100B and Mob1 might activate NDR family kinases by inducing a positive allosteric conformational change rather than by relieving an autoinhibited conformation. None the less, since mutations in the SMA domain affect the phosphorylation at the hydrophobic motif residue, it is also conceivable that SMA-interacting proteins, such as S100B or Mob1, mediate the targeting of an activating upstream kinase to NDR [11]. This idea is supported by the finding that Mob1 is essential for the phosphorylation of Dbf2 by its upstream kinase Ccd15 [8]. Significantly, we have recently found NDR1 autophosphorylation at the residue Thr-74 from the SMA domain. This residue proved to be crucial for the NDR1 activity in vivo; however, in contrast to Ser-281 and Thr-444, it seems to be involved solely in binding to S100B rather than regulating the activity of NDR1 per se. Of note, phosphorylatable residues serine or threonine are well conserved at this position in most NDR family kinases (except Dbf2 and 20, Sid2, and Cot-1 where the neighboring glutamates may, in part, substitute the negative charge of the phosphate moiety, see Fig. 2).

The functional interaction of NDR1 with Ca^{2+}/S100B indicated that Ca^{2+} levels may be an important factor in the regulation of NDR activity in vivo. In the presence of S100B, NDR1 becomes sensitive to changes in Ca^{2+} concentration in the 1–100 μM range, which corresponds well to the Ca^{2+} concentrations achieved in vivo and to the affinity of Ca^{2+} sensor S100B for these ions [4]. In fact, we found that the cell permeable Ca^{2+}-chelating agent 1,2-bis(*o*-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid tetra(acetoxymethyl)ester

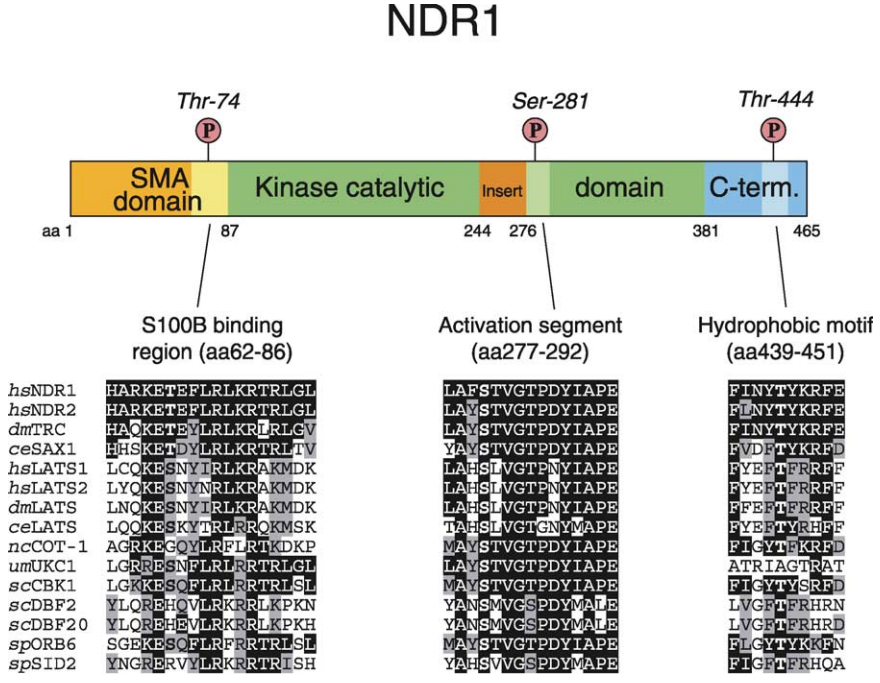

Fig. 2. Structure of NDR. The domain structure of NDR1 is shown including (i) the N-terminal regulatory SMA domain containing the S100B- and Mob1-binding region with the autophosphorylation residue Thr-74, (ii) the catalytic domain spliced by a 33 amino acid insert (encompassing a non-consensus NLS sequence) and containing the activation segment residue Ser-281, (iii) the C-terminal extension comprising the regulatory hydrophobic motif with the residue Thr-444. Below, conservation of the S100B-binding region, activation segment, and the hydrophobic motif among the members of NDR family is shown. The identical residues are boxed in black and similar residues are in gray. The phosphorylation sites are typed in bold.

(BAPTA-AM) suppresses the activity and phosphorylation of NDR1 on both Ser-281 and Thr-444, and specifically, these effects can be reversed upon addition of the sarcoplasmatic-endoplasmatic reticulum Ca^{2+} ATPase (SERCA) Ca^{2+} pump inhibitor thapsigargin [11]. However, since the treatment of cells with Ca^{2+}-ionophore A23187 or thapsigargin results only in a partial activation of NDR1, it seems likely that Ca^{2+} is necessary, but not sufficient, for the activity of NDR; i.e. an additional signal may be needed to achieve full NDR activation. Nevertheless, a novel, Ca^{2+}-dependent mechanism of NDR regulation can be predicted, involving the S100B-mediated stimulation of autophosphorylation on Ser-281 and the phosphorylation of Thr-444 by an as yet unidentified Ca^{2+}-dependent upstream kinase (Fig. 3).

A further level of NDR kinase family regulation has been discovered only very recently. Mutants in genes encoding a novel family of giant proteins of 300 kDa from yeast and *Drosophila*, Pag1, Mor2, and FRY, give rise to phenotypes that are indistinguishable from mutants in the corresponding NDR family kinases Cbk1, Orb6, and TRC, respectively [21–23]. They interact genetically, and at least for Cbk1 and Pag1, they also undergo a physical interaction. The similarity among the family members from different species is found in five mostly large domains separated by short regions of little or no similarity. Despite their length, the primary sequence of these proteins does not give any information about their function. Nevertheless, it seems likely that they may function as scaffolds recruiting other regulatory proteins and/or downstream targets, thereby organizing NDR signaling complexes in the different species.

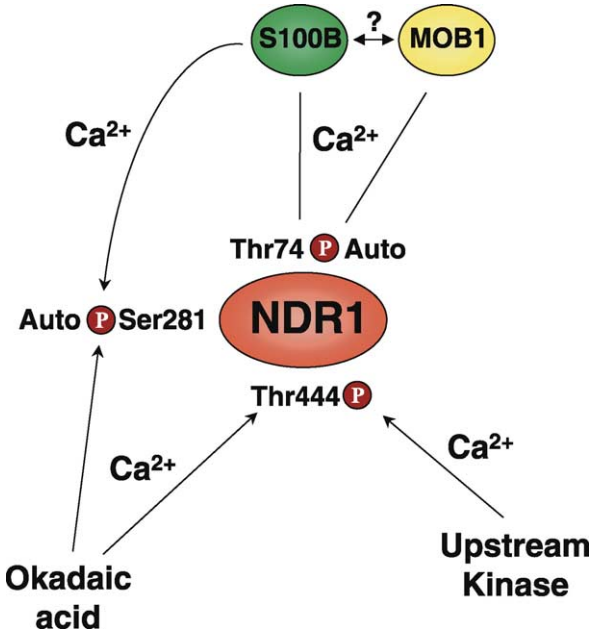

Fig. 3. Model of NDR regulation. NDR1 becomes autophosphorylated on the three indicated residues, Thr-74, Ser-281, and Thr-444, in vitro. The autophosphorylation and the kinase activity of NDR1 are promoted by an interaction with Ca^{2+}/S100B. The Ser-281 site is autophosphorylated also in vivo. In contrast, Thr-444 is targeted mainly by an as yet unidentified upstream kinase. Nevertheless, both phosphorylation events are Ca^{2+}-dependent and essential for the NDR1 activity in vivo. Thr-74 residue is crucial for the binding of S100B and Mob1, but it is not yet clear how its autophosphorylation influences the NDR1 activity in vivo. The mechanism of NDR activation through Mob1 and a possible interplay between S100B and Mob1 also remain to be elucidated.

3. In vivo function of NDR protein kinase family

3.1. S. cerevisiae *and* S. pombe

Cell growth and morphogenesis are complex processes that are tightly linked to the cell cycle and division. Substantial progress has been achieved in understanding how these intricate processes are coordinated in lower eukaryotes, in particular in the budding yeast *S. cerevisiae* and the fission yeast *S. pombe*. Numerous genetic, cytological and biochemical studies confirmed that the protein kinases of the NDR family

Table 1
Function of NDR family protein kinases

Kinase	Species	Function	References
Cbk1	*S. cerevisiae*	maintenance of polarized growth during budding and mating as well as cell wall remodelling after cytokinesis	[24-27]
Dbf2	*S. cerevisiae*	constituent of mitotic exit network (MEN) governing inactivation of CDKs and cytokinesis	[28-30]
Orb6	*S. pombe*	maintenance of polarized growth and control of mitosis by inactivating CDKs	[17, 33]
Sid2	*S. pombe*	a part of septation initiation network (SIN) that promotes formation of division septum and constitutes cytokinesis checkpoint	[34-38]
Cot-1	*N. crassa*	elongation and branching of hyphae	[39]
Ukc1	*U. maydis*	preservation of cell shape and control of hyphal and bud outgrowth	[40]
TRC	*D. melanogaster*	maintenance of integrity of cellular projections	[41]
SAX-1	*C. elegans*	control of cell shape and outgrowth of some types of neuritis	[46]
LATS1	*D. melanogaster* *C. elegans* mammals	control of cell morphology and proliferation; tumor suppressor negatively regulating cell proliferation through inhibition of CDK1 and promoting apoptosis through induction of BAX	[45, 47-51]
NDR1	mammals	a calcium-regulated kinase upregulated in some melanomas and breast ductal carcinoma *in situ*	[4, 53]

including *S. cerevisiae* Cbk1, Dbf2, and Dbf20 and *S. pombe* Orb6 and Sid2 are, along with their interacting proteins, key molecules orchestrating various aspects of cell morphogenesis and cytokinesis such as maintenance of cell shape and cell polarity, organization of actin and tubulin cytoskeleton, as well as the concerted completion of mitotic exit, septum formation, and daughter cell separation.

Inactivation of Cbk1, which is the closest NDR homologue in *S. cerevisiae*, results in a phenotype characterized by large aggregates of unseparated cells, round rather than ellipsoidal cells, diminished apical growth, and a random instead of bipolar budding pattern [24,25]. Cbk1 localizes to sites of polarized growth and cell wall remodeling, such as the actively expanding cell cortex during bud and mating projection formation, and later, moves to the bud neck and daughter cell nucleus during mitotic exit. This localization was shown to be dependent on two Cbk1-associating partners, Hym1 and Mob2 [25,26]. Mob2 is also required for Cbk1 kinase activity that changes during the cell cycle, peaking late in M phase and during the pheromone-induced G1 arrest. Interestingly, although F-actin organization is essentially normal in *cbk1*Δ and *mob2*Δ cells during vegetative growth, the exposure of these cells to mating pheromone (α factor) led to profound defects in formation of actin patches that concentrate at the tips of growing mating projections in wild-type cells, but are distributed evenly throughout the cell surface in pheromone-treated mutant cells. This suggests that the role of Cbk1 and Mob2 is the maintenance of persistent polarized growth in such structures. Furthermore, Cbk1 apparently regulates a broad range of genes encoding proteins with cell wall modification activities, such as *scw11* and *cts1* encoding glucanase and chitinase, respectively. Activity of these proteins is essential for efficient separation of mother and daughter cells after cytokinesis. At least the expression of Cts1, and perhaps also Scw1, is known to be controlled by Ace2, a daughter-specific transcription factor whose activity and proper localization to the nuclei of daughter cells were shown to depend on Cbk1 and Mob2 [27]. Collectively, the results suggest that Cbk1 regulates two distinct cell morphogenesis pathways: an Ace2-independent pathway that is required for polarized apical growth and mating projection formation and an Ace2-dependent pathway that is required for efficient cell separation following cytokinesis. It is also conceivable that Cbk1, together with Mob2, form a part of the 'cytokinesis checkpoint' which ensures that the cell wall degradation does not commence until membrane fission is completed.

Another NDR relative in budding yeast is the protein kinase Dbf2 and its close homologue Dbf20. Cells carrying temperature-sensitive alleles of *dbf2* arrest at restrictive temperature at the end of anaphase, with uniform large budded 'dumbbell' morphology, and display elongated mitotic spindles indicative of incomplete nuclear division [28]. The kinase activity of Dbf2 fluctuates during the cell cycle with maximum in anaphase, which coincides with the localization of Dbf2 to spindle pole bodies (SPBs or yeast centrosomes) [29,30]. Later in mitosis, immediately prior to actin medial ring assembly, Dbf2 migrates to the bud neck, consistent with its role in cytokinesis. Dbf2 associates and colocalizes with Mob1, a close homologue of Cbk1-interacting Mob2, and mRNAs for both Dbf2 and Mob1 are periodically expressed during the cell cycle peaking in mitosis. These two proteins constitute an integral part of mitotic exit network (MEN) that governs inactivation of mitotic cyclin-dependent kinases (CDKs) and cytokinesis (reviewed in [31]). MEN is a signaling complex that consists of the GTPase Tem1 with its two regulatory proteins, a two-component GTPase-activating protein (GAP) Bub2-Bfa1 and a guanine nucleotide-exchange factor (GEF) Lte1, polo-like kinase Cdc5, Dbf2 upstream kinase Cdc15 that associates with and activates Dbf2-Mob1, and finally, protein Nud1 that acts as a scaffold for Tem1, Bub2-Bfa1, Cdc15, and Dbf2 anchoring them on the SPB. The function of this cascade is to activate, and to release from nucleolar sequestration, the phosphatase Cdc14 which, in turn, inactivates the mitotic CDK Clb-Cdc28 through at least three different mechanisms (involving activation of cyclin degradation complex Cdh1-APC, stabilization of CDK inhibitor Sic1, and activation of transcription factor Swi5 driving the expression of Sic1). Dbf2 is genetically placed downstream of the other MEN components, but upstream of Cdc14. Since both Cdc14 and the nucleolar sequestering protein Cfi1/Net1 are known to be phosphorylated, it is conceivable that Dbf2 directly phosphorylates these proteins and mediates their dissociation [32].

In fission yeast, the closest homologue of NDR is the protein kinase Orb6, originally identified as one of 12 *orb* genes involved in cell morphogenesis [33]. Under normal growth conditions, the *S. pombe* cells switch in the early G2 phase to bipolar growth, using the new tip formed by cell division (new end take off, NETO). The bipolar growth continues until mitosis when tip elongation ceases, and the cell division commences. These cell cycle transitions are accompanied by profound changes in the actin cytoskeleton, involving formation of distinct actin patches at the growing tips in interphase and actin ring during mitosis. In the *orb* mutants, however, the cells become spherical and show a complete loss of cell polarity. Moreover, some of the mutants, including *orb6*, display disorganized microtubules as well as a random and delocalized distribution of F-actin throughout the cell cortex. Furthermore, decrease of Orb6 protein also results in advanced mitosis. On the other hand, Orb6 overexpression maintains polarized growth, but delays mitosis by affecting the mitotic CDK kinase Cdc2, possibly through the Wee1 protein kinase [17]. Orb6 protein is enriched at the cell tips during interphase and at the region of developing septum during mitosis and cytokinesis, emphasizing the role of this kinase in the maintenance of polarized growth and cell division. Genetic data suggest that the correct localization of Orb6 depends on protein kinase Pak1 (also known as Shk1 or Orb2), an effector of the GTPase Cdc42. Similar to its *S. cerevisiae* homologue Cbk1, Orb6 physically interacts and colocalizes with Mob2, and the inactivation or overexpression of Mob2 results in identical phenotypes as those observed for Orb6. Thus, Orb6 may act downstream of Pak1, forming a part of a signaling pathway coordinating cell morphogenesis with the progression through cell cycle.

The fission yeast counterpart of Dbf2 is protein kinase Sid2 that was originally isolated as a mutant defective in formation of septum during cytokinesis [34,35]. Together with its binding partner Mob1 (an ortholog of *S. cerevisiae* Mob1), it forms an effector part of *S. pombe* septation initiation network (SIN) that resembles, yet is not identical to the *S. cerevisiae* MEN system [36]. SIN consists of GTPase Spg1, two-component GAP Cdc16-Byr4, SPB-anchoring proteins Sid4 and Cdc11, protein kinases Plo1, Cdc7, as well as Sid1 and Sid2 with their

associating factors Cdc14 (unrelated to its *S. cerevisiae* namesake) and Mob1, respectively (reviewed in [31]). The SIN components localize at SPBs throughout mitosis, but are inactive until formation of the metaphase spindle, which triggers dissociation of the Cdc16-Byr4 complex, and thereby, activation of Spg1 and recruitment of Ccd7 to SPBs. During anaphase, Mob1-Sid2 kinase is activated in a Ccd7- and Cdc14-Sid1-dependent manner, relocalizes to the medial ring and flanks the septum as medial ring contracts [36,37]. Since the Sid2 and Mob1 are the only SIN pathway components seen at the site of the division septum, it is thought that these proteins transmit the executive message for septum formation to the site of cytokinesis. In addition, Mob1-Sid2 and the other SIN pathway members could also be involved in 'cytokinesis checkpoint' that acts as a safeguard preventing cells with unassembled division septum to enter new cell cycle [38]. This system is not well understood yet, but it is known that it acts through Clp1/Flp1, a *S. pombe* counterpart of the budding yeast Ccd14, which can inhibit the mitotic CDK Cdc13-Cdc2 via activation of Wee1 kinase, inhibition of Cdc25 phosphatase, or through promoting both events. Clp1/Flp1 is normally kept inactive by its sequestration in the nucleolus, and after its delivery, an active SIN is needed to maintain Clp1/Flp1 in its released state [38].

Examples from other fungal organisms further illustrate the role of NDR family kinases in control of morphogenesis. A mutation in *cot-1* gene, which encodes the NDR homologue Cot-1 in filamentous fungi *N. crassa*, results in restricted colonial growth with formation of small and compact colonies [39]. This growth alteration is due to a defect in hyphal tip elongation and a concomitant increase in hyphal branching, suggesting that Cot-1 kinase is required for one or more events essential for hyphal outgrowth. On the other hand, the disruption of *ukc1* gene encoding NDR family member Ukc1 in the bimorphic fungi *U. maydis* is manifested by highly distorted cell morphology, inability to generate aerial filaments during mating, and compromised pathogenicity. This mutant forms clusters composed of rounded cells with hyphal extensions or elongated buds resembling chlamydospore-like cells [40].

3.2. D. melanogaster

The *D. melanogaster* gene *tricornered* (*trc*) encodes the protein kinase TRC displaying 70% identity and 79% similarity to the human NDR1 kinase throughout the whole protein sequence, indicating that these two proteins are orthologs. A mutation in *trc* is associated with organismal recessive lethality, but it is still cell viable, and has been used as a gratuitous cuticular marker in a number of genetic mosaic studies. Significantly, mutations in *trc* result in the splitting, clustering, or branching of a variety of polarized structures such as epidermal hairs decorating the adult cuticular surface, the shafts of bristle sense organs, the lateral extensions of the arista, and the larval denticles ([41] and references therein). The morphology and polarity of branched hairs, laterals, or bristles is, however, typically normal except for the region of the branch point. In this respect, *trc* differs fundamentally from mutations in the downstream components of *frizzled* tissue polarity pathway such as *inturned* or actin cytoskeleton components such as *crinkled*, *singed*, or *forked* which exhibit an altered polarity of cellular projections or abnormal morphology in all regions of the structure (reviewed in [42]. Nevertheless, inhibition of actin polymerization in differentiating cells by cytochalasin D or latrunculin A treatment was found to phenocopy the branched hairs and bristles found in *trc*, suggesting that TRC might interact with actin cytoskeleton. Consistent with this possibility, Geng et al. [41] found that a weak *trc* mutant was hypersensitive to cytochalasin D treatment. However, the drugs also result in short, fat, and occasionally malformed hairs and bristles. Such differences between phenotypes induced by *trc* mutant and the drug treatment suggest that TRC is not simply required for actin polymerization, but it seems more likely that TRC interacts with the actin cytoskeleton in a more subtle way. There are at least three possible mechanisms for the action of TRC. It might form a part of molecular machinery monitoring and coordinating the growth of actin filaments and bundles during the outgrowth of cellular projections, the so called 'morphogenetic checkpoint' [43]. Alternatively, TRC protein could function in the assembly of the cellular components that initiate and organize the outgrowth of cellular extensions. Defects in proper assembly of such initiation centers, as well as formation of multiple or too large initiation centers, might result in the splitting of the extensions at later stages of elongation. Finally, TRC could also function to mechanically strengthen the cellular projections to ensure their integrity, e.g. by increasing the degree of cross-linking of membrane cytoskeleton.

A close NDR homologue, LATS/WARTS, was first identified in mosaic fly screens for tumor suppressors and negative regulators of cell proliferation [44]. Somatic cells from *lats*-negative flies undergo extensive proliferation and form large tumors in many tissues in mosaic adults. Moreover, homozygous mutants for various *lats* alleles display a range of developmental defects including embryonic lethality. The *lats* mutant clones induced at several developmental stages form dramatically overproliferated tissues in adults. Cells in these clones are capable of differentiation on the fly body and produce bristles and hairs, but the morphology of these extensions and the cell shape are altered compared to wild-type cells [45].

3.3. C. elegans

The *C. elegans* gene *sax-1* encodes protein kinase with 66% identity and 74% similarity to human NDR1. *Sax-1* mutants exhibit defects in neuronal cell shape and polarity. The cells have expanded to irregular cell bodies and initiate ectopic neurites in several classes of sensory neurons, suggesting that SAX-1 functions to restrict cell and neurite growth [46]. The ectopic neurites resemble the effect of decreased sensory activity in nematodes with mutations in the cGMP-gated sensory transduction channel TAX-4. However, the neurons in the *sax-1*/*tax-4* double mutant display significantly more neurites than a null mutant in either gene, indicating that these genes function in two different pathways to suppress neurite initiation. Similarly, mutation in *unc-43* gene encoding the *C. elegans* CaM-dependent kinase II (CaMKII), which has been implicated in the regulation of neuronal morphology in response to neural activity, leads to far more severe phenotype in double mutants with *sax-1* (but not with *tax-4*). None the less, the elevated activity of UNC-43 in part suppresses the *sax-1* mutant, suggesting that UNC-43 can partially compensate for the decreased SAX-1 kinase activity. Thus, the activity-dependent pathway for neurite initiation is modulated by UNC-43 CaMKII kinase, and functions in parallel with

SAX-1 kinase. Of note, mutations in the GTPase RhoA produce a similar phenotype in sensory neurons as the *sax-1* mutants, although the defects are less marked than those in *sax-1* worms. Most significantly, the *sax-1*/*rhoA* double mutant strain has defects that are no more severe than the *sax-1* single mutant, suggesting that SAX-1 and RhoA affect a similar process. In agreement with this idea, overexpression of *sax-1* partly suppresses the *rhoA* defects and vice versa, pointing to the fact that SAX-1 and RhoA may be components of the same morphogenetic pathway or two pathways with shared functions.

3.4. Mammals

The best studied member of NDR kinase family in mammals is the protein kinase LATS1. It has been shown to localize to the centrosomes in interphase, to translocate to the mitotic spindle in metaphase and anaphase, and then to the midbody in telophase [47]. Furthermore, it is phosphorylated in a cell cycle-dependent manner, and interacts directly with CDK1 in early mitosis. LATS1-associated CDK1 has no mitotic cyclin partner and no kinase activity, indicating that LATS1 is a novel negative regulator of CDK1 [48]. Transduction of many human tumor cell lines with LATS1 by an adenovirus-mediated gene transfer brings about a marked inhibition of cell proliferation. Moreover, ectopic expression of LATS1 specifically downregulates the cyclin A and cyclin B protein levels, and dramatically reduces CDK1 activity, leading to growth arrest in the G2/M phase. It also can induce the level of BAX protein, thereby promoting apoptosis [49,50]. Abolition of LATS1 function in LATS1$^{-/-}$ mice leads to impaired mammary gland development, infertility, growth retardation, and most significantly, to the development of soft tissue sarcomas and ovarian stromal cell tumors [51]. Taken together, these observations suggest that LATS1 is a tumor suppressor that suppresses tumorigenesis by negatively regulating cell proliferation and modulating cell survival.

Less is currently known about the in vivo function of NDR1 and NDR2 protein kinases. However, a number of observations suggest that mammalian NDR may perform similar functions as its relatives in other species. For instance, NDR interacts with hMob1 which is the human homologue of Mob1 and Mob2 from *S. pombe* and *S. cerevisiae* (S.J. Bichsel et al., manuscript in preparation). This indicates that the regulation of NDR may be analogous to the members of NDR family in yeast. Moreover, activity of NDR appears to be tightly cell cycle regulated also in mammalian cells (our unpublished observations). In fact, the current data indicate that NDR can be involved in control of cell proliferation and tumor development. First, the NDR-activating protein S100B is overexpressed in $\geqslant$80% of metastatic melanomas, and serum levels of S100B are used as a prognostic marker for tumors with poor prognosis [52]. Significantly, NDR is hyperactivated in some S100B-positive melanoma cell lines, and inhibitor studies suggest that S100B is the direct cause for this elevated NDR activity [4]. Second, NDR1 mRNA level was found to be consistently upregulated in the ductal carcinoma in situ (DCIS) that marks high risk of invasive breast cancer [53]. Third, both NDR1 gene location at the chromosome locus 6p21 and the NDR2 gene from 12p11 belong to regions that were described as cancer amplicons ([54] and references therein). These data suggest that NDR, along with its partners S100B or hMob1, may form a part of novel calcium-dependent signaling pathway that controls cell proliferation, and its deregulation is involved in pathogenesis of some malignant disorders.

4. Future perspectives

Elucidation of the NDR signaling pathway in higher organisms is expected to provide further insights into the control of cell division and morphogenesis. The example of tumor suppressor LATS1 clearly illustrates the importance of NDR family kinases in growth control in metazoans. The novel methods of systematic identification of protein complexes by means of high-throughput mass spectrometric protein complex identification (HMS-PCI) and of identification of suppressors of epistatic mutations by the synthetic genetic array mapping (SGAM) have recently greatly illuminated the function of the Cbk1 pathway in budding yeast. For instance, this signaling pathway was suggested to participate, through the genetic and/or physical interactions of Cbk1 with proteins such as SEC28, VMA6, BST1, GOS1 or SSD1, also in the control of vesicular transport or RNA stability [55,56]. Thus, it seems likely that, in addition to their role in diverse aspects of cell division and morphogenesis, NDR family protein kinases are involved in more cellular processes than previously assumed. Future research will be certainly focused on investigations of such novel functions of these kinases. Finally, although enormous progress has been achieved in characterization of function and components of the NDR kinase family signaling, the specific protein substrates targeted by these kinases still remain to be identified.

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