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Role for DYRK family kinases on regulation of apoptosis

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ABSTRACT

The cellular response to a variety of stress including DNA damage is involved in cell cycle arrest, activation of DNA repair, and in the event of irreparable damage, induction of apoptosis. However, the signals that determine cell fate, that is, survival or apoptosis, are largely unknown. Accumulating studies have revealed that dual-specificity tyrosine-regulated kinases (DYRKs) play key roles on cell proliferation and apoptosis induction. In particular, DYRK2 translocates from the cytoplasm into the nucleus following genotoxic stress. DYRK2 is then activated by ATM and induce apoptosis by phosphorylating p53 at Ser46. Importantly, whereas precise regulation of these kinases remain uncertain, this mechanism has consequences for cell proliferation, differentiation, or apoptosis. This progress review highlights recent efforts demonstrating that DYRKs could be novel and essential regulatory molecules for the regulation of cell fate including apoptosis.

1. Introduction

Dual-specificity tyrosine-regulated kinases (DYRKs) are a novel subfamily of protein kinases that catalyze their autophosphorylation on tyrosine residues and the phosphorylation of serine/threonine residues on exogenous substrates [1–3]. Their kinase activity depends on the presence of a YXY motif in the activation loop of the catalytic domain [4], which is located at the similar position as the characteristic TXY motif of the mitogen-activated protein kinases (MAPKs). This suggests a potential involvement of these proteins in signal transduction pathways similar to those of the MAPKs [5], although, unlike MAPKs, Tyr phosphorylation in the DYRK activation loop is an autophosphorylation event and does not involve an upstream activating kinase [3]. Indeed, phosphorylation of the second Tyr residue of this motif is essential for the kinase activity of all the DYRK members reported so far [3,6,7].

Phylogenetic analysis suggests that DYRKs are classified into three subfamilies (Fig. 1). Lower eukaryotic members of this family are the kinases Pom1p in Schizosaccharomyces pombe [8], Yak1p in *Saccharomyces cerevisiae* [9], and YakA in Dictyostelium discoideum [10]. The DYRK1 subfamily is animal specific and represented by the mammalian DYRK1, minibrain (mnb) in Drosophila melanogaster, and *C. elegans* MBK1. On the other hand, the DYRK2 subfamily is conserved among diverse eukaryotic organisms, including mammalian DYRK2, fly's smi35A, and worm's MBK2. Interestingly, no member of the DYRK2 subfamily is found in budding yeast species, whose cell polarity is established by F-actin independently of microtubules [11]. Absence of a DYRK2 member in budding yeast might imply the function of the DYRK2 subfamily in the microtubule-driven cell polarity in other eukaryotes, as has been found with Pom1 [8]. Although strains with mutations in these proteins present different phenotypic abnormalities, all of them seem to be involved in cell cycle regulation and the control of cell proliferation and differentiation. The DYRK protein of Drosophila, mnb, is implicated in postembryonic neurogenesis. Mutant flies with down-regulation of mnb expression present reductions in the volumes of the adult optic lobes and central brain hemispheres, mainly due to abnormal spacing of neuroblasts in the outer proliferation center of the larval brain [12]. Mammalian DYRKs include DYRK1A, DYRK1B (or MIRK), DYRK2, DYRK3, and DYRK4 [13] (Fig. 1). DYRK1A is the most

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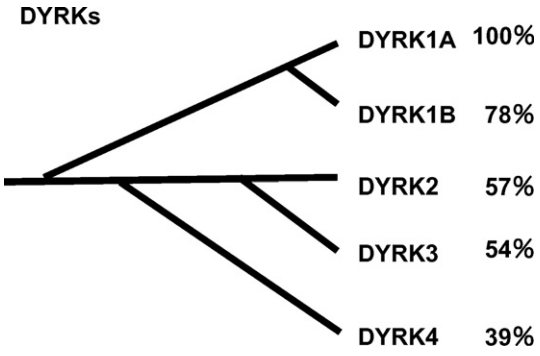

Fig. 1 – A phylogenetic tree for the kinase domain sequences of DYRKs. % homology was defined according to the comparison with DYRK1A sequence.

extensively characterized member of this family, and it shares all the characteristic motifs of the catalytic domain with the other family members. Outside the catalytic domain, the sequence comprises several striking structural features, such as a bipartite nuclear localization signal, a PEST domain, a histidine repeat, and a region rich in serine-threonine residues [4].

2. DYRK1A

DYRK1A is the human orthologue of the Drosophila mnb gene, which is involved in postembryonic neurogenesis in flies. Because of its mapping position on chromosome 21 and the neurobehavioral alterations shown by mice overexpressing this gene, it has been suggested that DYRK1A is involved in some of the neurological defects of Down syndrome (DS) patients. The human DYRK1A gene maps to chromosome 21 (HSA21) in the DS critical region 21q22.2 [14–16]. Part of this region includes the chromosomal segment deleted in HSA21-linked microcephaly [17]. The mouse DYRK1A gene maps to chromosome 16, in the region of synteny with HSA21 [18]. The human DYRK1A and rodent DYRK1A genes are ubiquitously expressed in tissues of adult and fetal origin [14,16]. In addition, DYRK1A is overexpressed in DS fetal brains, while its mouse orthologue is overexpressed in the brains of adult Ts65Dn mice [19], a partial trisomy 16 mouse model, widely used as a model for DS [20]. These data collectively indicate that DYRK1A might be one of the genes involved in some of the neurological abnormalities observed in DS patients. In agreement with this is the fact that transgenic mouse models overexpressing the DYRK1A gene present a deficit in visuospatial learning and memory [21,22]. Heterozygous DYRK1A mice also present a noticeable phenotype, with region-specific brain alterations [23]. Another seminal study demonstrated that, by using embryonic rats, DYRK1A is strongly expressed in brain and heart at the early postnatal stage [24]. Interestingly, DYRK1A expression was gradually decreased with postnatal growth. In this context, children with DS have normal brain weights at birth, however, the weights become significantly lower than normal by late childhood [25]. Furthermore, congenital anomaly of the heart often occurs in DS children. Taken together, these findings strongly suggest that DYRK1A plays a pivotal role on the development of brain and heart.

DYRK1A harbors, in addition to the conserved catalytic kinase domain, two nuclear localization signals (NLSs): a classical bipartite NLS at the N terminus and a complex NLS within the catalytic domain [26]. The kinase domain is followed by a PEST domain and then by a repeat of histidines that targets DYRK1A to the splicing factor compartment [26]. DYRK1A has been defined as a dual-specificity kinase because of its ability to autophosphorylate on Tyr and Ser/Thr residues. Moreover, in exogenous substrates it can only phosphorylate Ser and Thr residues that occur within a consensus phosphorylation sequence, RPX(S/T)P, and with a preference for proline in the +1 position [3,27]. Substrates of DYRK1A, some of them awaiting confirmation in vivo, have been identified among different classes of proteins. DYRK1A phosphorylates a variety of substrates in vitro, such as the signal transducer and activator of transcription 3 (STAT3) [28], the subunit of eukaryotic initiation factor 2B (eIF2B), the microtubule-associated protein tau [29], Gli1 [30], the transcription factor of the forkhead family FKHR [31], several splicing factors, including cyclin L2 [32] and SF3b/SAP155 [33]; and some cytosolic molecules such as dynamin [34] and the enzyme glycogen synthase [35]. These data clearly indicate its potential involvement in various biochemical pathways in vivo. A recent report on the Drosophila DYRKs mnb and dDYRK2 shows that autophosphorylation in the activation loop of these kinases is an intramolecular event, mediated by a transitional intermediate form during translation [36]. The tyrosine kinase activity is lost once the protein is fully translated, and the mature kinase can only phosphorylate Ser/Thr residues. Assuming this mechanism to be general for all DYRKs, the autophosphorylation of the activation loop in DYRK1A would be constitutive. However, some DYRK1A functions are clearly dose and activity dependent [37,38], and it is therefore likely that the activity of DYRK1A will be regulated by additional mechanisms. In this context, recent findings demonstrated that the catalytic activity of DYRK1A is regulated by autophosphorylation and binding to 14-3-3 protein [39,40]. This interaction induces its conformational change, resulting in increased DYRK1A catalytic activity.

The available data with in vivo role demonstrated that DYRK1A, when activated by the basic fibroblast growth factor (bFGF) in immortalized hippocampal progenitor cells, stimulates the phosphorylation of the cyclic AMP response element binding protein (CREB) and induces subsequent CRE-mediated gene transcription. In addition, overexpression of a kinase-deficient DYRK1A remarkably attenuated the differentiation of hippocampal cells [41]. Whereas the exact role for DYRK1A in central nervous system (CNS) function has not been determined, this recent finding provides the first evidence indicating the involvement of DYRK1A in neuronal differentiation [41].

3. DYRK1B/MIRK

MIRK/DYRK1B is a member of the Minibrain/DYRK family of kinases [4,12,13] which mediate survival and differentiation in certain normal tissues, including skeletal muscle [42], and that

play pivotal roles in controlling the switch from proliferation to differentiation in a wide variety of organisms. MIRK is expressed at elevated levels in skeletal muscle compared to other normal tissues [43]. The skeletal muscle maturation program is controlled by the basic helix-loop-helix (bHLH) transcription factor MyoD which is activated by the GTPase RhoA. RhoA also induces MIRK expression together with bHLH factors through an E-box site mapped in the MIRK promoter, enabling MIRK expression to up-regulate about 10-fold during differentiation [44]. MIRK depletion blocks myoblast differentiation into multinucleated myotubes, while stable expression of MIRK increases their rate of fusion [44]. MIRK has transcriptional activator activity for HNF1α [45] and also for the bHLH transcription factor MEF2, which is active in myoblast differentiation [46]. MEF2 is sequestered by class II histone deacetylases (HDACs). MIRK phosphorylates these class II HDACs at a conserved site within their nuclear localization region, inhibiting their nuclear accumulation in a dose-dependent and kinase-dependent manner. The reduced HDAC concentration in the nucleus releases MEF2 to transcribe myogenin [46] and enables differentiation to sustain. MIRK functions to promote cell survival during the initial stages of differentiation [42]. MIRK diminishes the extent of myoblast apoptosis during the differentiation process, at least in part by direct modulation of $p21^{Cip1}$ localization. MIRK phosphorylates $p21^{Cip1}$ around its nuclear localization domain. This phosphorylation maintains a portion of $p21^{Cip1}$ protein in the cytoplasm where $p21^{Cip1}$ is unable to mediate cell cycle arrest [47] and where $p21^{Cip1}$ blocks caspase-3 activation [48].

4. DYRK2

The DYRK family is characterized by the presence of several distinct amino-acid sequences in the kinase domain, including an SSC motif following subdomain VII, conserved sequences HCDLKPEN and YXYIQSRFYR(S/A)PE in subdomains VI and VIII, respectively, and a YXY motif in the kinase-domain-activation loop between subdomains VII and VIII, and by a DYRK homology (DH) box immediately preceding the kinase domain (Fig. 2) [13]. In this context, DYRK2 shares a conserved kinase domain and adjacent N-terminal DH box but does not contain a C-terminal PEST domain (Fig. 2). DYRK2 is presumed to be involved in regulating key developmental and cellular processes such as neurogenesis, cell proliferation, cytokinesis and cellular differentiation. However, substrates for DYRK2 by serine/threonine phosphorylation are little

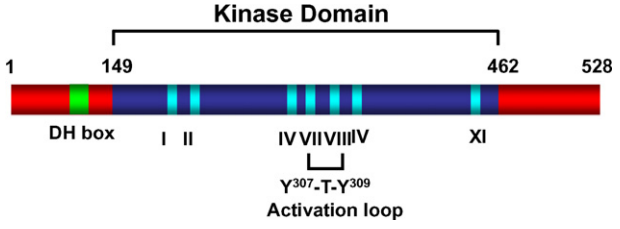

Fig. 2 – A schematic structure of DYRK2. Roman numerals designate the kinase subdomains according to the nomenclature of Hanks [59]. DH-box, DYRK homology box.

Table 1 – DYRK2 substrates with phosphorylation sites

DYRK2 Substrates	Phosphorylation sites
Stat3 (Ser^{727})	LPMSP
tau (Thr^{212})	RSRTP
eIF2Bε (Ser^{539})	RAGSR
NFAT (Drosophila)	SPQRSRSPSPQPSPH
SNR1 (Drosophila) (Thr^{102})	TSDTP
p53 (Ser^{46})	LMLSP

determined (Table 1). Recent findings have shown that DYRK1A and DYRK2 phosphorylate NFATc, which regulates calcium signaling, to lead NFATc inactivation by its cytoplasmic sequestration [37,38]. In addition to calcium signaling, another study demonstrated that, in the fission yeast *Schizosaccharomyces pombe*, the DYRK-family member Pom1 kinase is involved in a cell-polarity maintenance under stress conditions, such as heat-shock or high osmolarity [49]. Interestingly, MIRK is overexpressed in primary colon carcinomas and can mediate the survival of colon carcinoma cell lines under stress-induced conditions [43]. A previous study has reported that DYRK2 mRNA was down-regulated in neuroblastoma cells by treating the cells with an anticancer agent, 8-Cl-cAMP, a site-selective cyclic AMP analogue exhibiting growth inhibition in a broad spectrum of human cancer lines [50]. These observations indicate a potential role for DYRK2 in tumor development and/or progression. However, to our knowledge, involvement of DYRKs in other stress responses including DNA damage is unknown.

Upon exposure to genotoxic stress, p53 is stabilized and activated by phosphorylation at Ser15 and Ser20 to regulate a cell cycle checkpoint and DNA repair. In case of the lesion for irreparable DNA damage, p53 induces apoptotic cell death by a mechanism in which an additional phosphorylation increases the binding affinity of p53 to promoters of pro-apoptotic genes, such as p53AIP1. In this context, previous studies have established the mechanism in which p53 transactivates p53AIP1 by its additional phosphorylation at Ser46, thereby this phosphorylation is essential for p53-dependent apoptosis [51,52]. Ser46 kinase(s) thus functions in p53-dependent apoptosis; however, little was known of the kinase(s) responsible for Ser46 phosphorylation. We recently demonstrated that DYRK2 is a novel Ser46 kinase (Fig. 3) [53]. Our data show that DYRK2 has the characteristics of an in vitro direct Ser46 kinase. Moreover, our results demonstrate that DYRK2 phosphorylates p53 at Ser46 in cells exposed to genotoxic stress. Significantly, DYRK phosphorylation of Ser46 was associated with the induction of apoptosis following DNA damage (Fig. 3). These findings provide support a novel signaling mechanism in which phosphorylation of p53 at Ser46 by DYRK2 regulates apoptotic cell death in response to DNA damage.

A previous study demonstrated that DYRK2 is predominantly expressed in the cytoplasm [13]. We confirmed the cytoplasmic localization of DYRK2 in unstimulated cells. Furthermore, we found that DYRK2 translocates from the cytoplasm into the nucleus in response to DNA damage. The mechanism for nuclear targeting of DYRK2 is, at present, not known. There was little if any difference between control cells and ATM silenced cells on DNA damage-induced nuclear

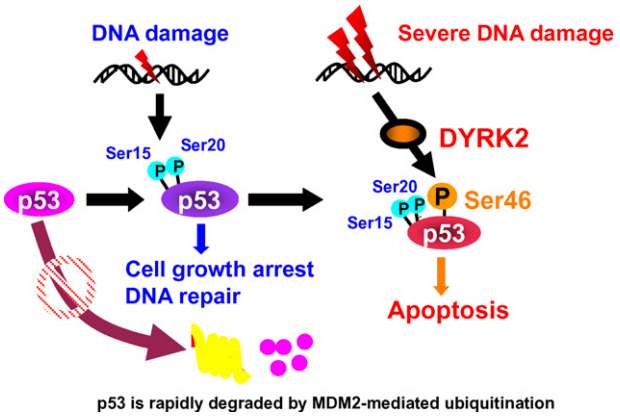

Fig. 3 – Sequential phosphorylations and distinct functions of p53 in response to DNA damage. In unstressed condition, p53 is rapidly degraded by MDM2-mediated ubiquitination and proteasome system. Upon exposure to DNA damage, p53 is phosphorylated at Ser15 and Ser20 to escape from degradation by inhibiting interaction with MDM2. Phosphorylated p53 induces cell cycle arrest or DNA repair. If cells are exposed severe DNA damage, p53 is additionally phosphorylated at Ser46 by DYRK2 to strongly induce apoptotic cell death by transactivating p53AIP1.

targeting of endogenous DYRK2, suggesting that nuclear translocation of DYRK2 is independent of its activity. Importantly, however, nuclear translocation may be required for efficient phosphorylation of p53 at Ser46 and, therefore it is conceivable that Ser46 phosphorylation occurs in relatively later periods following genotoxic stress [51,52]. Indeed, our findings that nuclear re-distribution of DYRK2 coincides with Ser46 phosphorylation of nuclear p53 further support the mechanism in which nuclear targeting of DYRK2 is, at least in part, required for sufficient phosphorylation of p53 at Ser46 in the nucleus [53].

5. DYRK3

DYRK3 is selectively expressed at high levels in hematopoietic cells of erythroid lineage. Using an antisense oligonucleotide approach, it has been demonstrated, in primary murine and human hematopoietic progenitor cells, that inhibition of DYRK3 expression significantly and specifically affects the production of colony-forming units-erythroid (the penultimate progenitors of erythroblasts) [54,55]. DYRK3 activity is shown to depend upon intactness of Tyr333 within its predicted autophosphorylation loop, and loop acidification is proved to be activating. This is unlike ERK2, for example, which possess an equivalently positioned TXY loop, but is not affected markedly by tyrosine acidification [56]. DYRK3 is shown to act via kinase domain- as well as unique C-terminal domain-dependent mechanisms to regulate CREB and CRE response pathways via PKA-dependent routes. DYRK3 expression in hematopoietic progenitor cells is revealed to modulate apoptosis due to cytokine withdrawal. DYRK3 has been shown to attenuate apoptosis due to cytokine withdrawal in IL-3-dependent hematopoietic FDCW2 cells, and this occurred independently of any detectable effect on mitogenic potential [6]. Roles for DYRKs in cell survival have not previously been well studied or described.

6. DYRK4

In contrast to well-studied DYRK1A, 1B, 2, and 3 isoforms, barely no data, apart from its raw sequence, are available for DYRK4. A recent study demonstrated a crucial role of DYRK4 in spermiogenesis based on its highly restricted testicular expression pattern [57]. A DYRK4-deficient mouse line was analyzed with respect to the potential involvement of the kinase in spermiogenesis and sperm function. However, DYRK4 deficient mice are fertile without any obvious dysfunctions in spermatogenesis, sperm motility and fertilization. Obviously, further studies are needed to get more insight into the potential cellular function of the DYRK4.

7. Perspectives

The homeodomain-interacting protein kinases (HIPKs) belong to the larger family of the DYRKs and consequently are closely related to DYRKs [1]. Interestingly, it has been shown recently that even though HIPK1 and HIPK2 single deficient mice are viable and fertile, HIPK1 and HIPK2 double deficiency leads to an embryonic mortality at mid-gestation [58]. This indicates that members of the DYRK superfamily do act in a functional redundant manner. In this context, the molecular bases are still unclear and future work has to face the open question how the DYRKs are compensatively involved in cell growth, differentiation, and apoptosis. In this respect should the future analyses of single DYRK as well as double deficient mice provide a more detailed view on the synergistic action of the DYRK kinases and their contribution to cell regulation. Finally, a more detailed analysis of the DYRKs-dependent signal transduction cascade including a comprehensive screen for putative substrates should give more insight into the molecular function of DYRKs.

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REFERENCES

[1] Becker W, Joost HG. Structural and functional characteristics of Dyrk, a novel subfamily of protein kinases with dual specificity. Prog Nucleic Acid Res Mol Biol 1999;62:1–17.
[2] Campbell LE, Proud CG. Differing substrate specificities of members of the DYRK family of arginine-directed protein kinases. FEBS Lett 2002;510:31–6.
[3] Himpel S, Panzer P, Eirmbter K, Czajkowska H, Sayed M, Packman LC, et al. Identification of the autophosphorylation sites and characterization of their

effects in the protein kinase DYRK1A. Biochem J 2001;359:497–505.
[4] Kentrup H, Becker W, Heukelbach J, Wilmes A, Schurmann A, Huppertz C, et al. Dyrk, a dual specificity protein kinase with unique structural features whose activity is dependent on tyrosine residues between subdomains VII and VIII. J Biol Chem 1996;271:3488–95.
[5] Miyata Y, Nishida E. Distantly related cousins of MAP kinase: biochemical properties and possible physiological functions. Biochem Biophys Res Commun 1999;266:291–5.
[6] Li K, Zhao S, Karur V, Wojchowski DM. DYRK3 activation, engagement of protein kinase A/cAMP response element-binding protein, and modulation of progenitor cell survival. J Biol Chem 2002;277:47052–60.
[7] Lochhead PA, Sibbet G, Kinstrie R, Cleghon T, Rylatt M, Morrison DK, et al. dDYRK2: a novel dual-specificity tyrosine-phosphorylation-regulated kinase in Drosophila. Biochem J 2003;374:381–91.
[8] Bahler J, Pringle JR. Pom1p a fission yeast protein kinase that provides positional information for both polarized growth and cytokinesis. Genes Dev 1998;12:1356–70.
[9] Garrett S, Menold MM, Broach JR. The *Saccharomyces cerevisiae* YAK1 gene encodes a protein kinase that is induced by arrest early in the cell cycle. Mol Cell Biol 1991;11:4045–52.
[10] Souza GM, Lu S, Kuspa A. YakA a protein kinase required for the transition from growth to development in Dictyostelium. Development 1998;125:2291–302.
[11] Pruyne D, Bretscher A. Polarization of cell growth in yeast I. Establishment and maintenance of polarity states. J Cell Sci 2000;113(Pt 3):365–75.
[12] Tejedor F, Zhu XR, Kaltenbach E, Ackermann A, Baumann A, Canal I, et al. Minibrain: a new protein kinase family involved in postembryonic neurogenesis in Drosophila. Neuron 1995;14:287–301.
[13] Becker W, Weber Y, Wetzel K, Eirmbter K, Tejedor FJ, Joost HG. Sequence characteristics, subcellular localization, and substrate specificity of DYRK-related kinases, a novel family of dual specificity protein kinases. J Biol Chem 1998;273:25893–902.
[14] Guimera J, Casas C, Pucharcos C, Solans A, Domenech A, Planas AM, et al. A human homologue of Drosophila minibrain (MNB) is expressed in the neuronal regions affected in Down syndrome and maps to the critical region. Hum Mol Genet 1996;5:1305–10.
[15] Shindoh N, Kudoh J, Maeda H, Yamaki A, Minoshima S, Shimizu Y, et al. Cloning of a human homolog of the Drosophila minibrain/rat Dyrk gene from ''the Down syndrome critical region'' of chromosome 21. Biochem Biophys Res Commun 1996;225:92–9.
[16] Song WJ, Sternberg LR, Kasten-Sportes C, Keuren ML, Chung SH, Slack AC, et al. Isolation of human and murine homologues of the Drosophila minibrain gene: human homologue maps to 21q22 2 in the Down syndrome ''critical region''. Genomics 1996;38:331–9.
[17] Matsumoto N, Ohashi H, Tsukahara M, Kim KC, Soeda E, Niikawa N. Possible narrowed assignment of the loci of monosomy 21-associated microcephaly and intrauterine growth retardation to a 1 2-Mb segment at 21q22. 2. Am J Hum Genet 1997;60:997–9.
[18] Song WJ, Chung SH, Kurnit DM. The murine Dyrk protein maps to chromosome 16, localizes to the nucleus, and can form multimers. Biochem Biophys Res Commun 1997;231:640–4.
[19] Guimera J, Casas C, Estivill X, Pritchard M. Human minibrain homologue (MNBH/DYRK1): characterization, alternative splicing, differential tissue expression, and overexpression in Down syndrome. Genomics 1999;57:407–18.
[20] Davisson MT, Schmidt C, Akeson EC. Segmental trisomy of murine chromosome 16: a new model system for studying Down syndrome. Prog Clin Biol Res 1990;360:263–80.
[21] Altafaj X, Dierssen M, Baamonde C, Marti E, Visa J, Guimera J, et al. Neurodevelopmental delay, motor abnormalities and cognitive deficits in transgenic mice overexpressing Dyrk1A (minibrain), a murine model of Down's syndrome. Hum Mol Genet 2001;10:1915–23.
[22] Smith DJ, Stevens ME, Sudanagunta SP, Bronson RT, Makhinson M, Watabe AM, et al. Functional screening of 2 Mb of human chromosome 21q22 2 in transgenic mice implicates minibrain in learning defects associated with Down syndrome. Nat Genet 1997;16:28–36.
[23] Fotaki V, Dierssen M, Alcantara S, Martinez S, Marti E, Casas C, et al. Dyrk1A haploinsufficiency affects viability and causes developmental delay and abnormal brain morphology in mice. Mol Cell Biol 2002;22:6636–47.
[24] Okui M, Ide T, Morita K, Funakoshi E, Ito F, Ogita K, et al. High-level expression of the Mnb/Dyrk1A gene in brain and heart during rat early development. Genomics 1999;62:165–71.
[25] Becker L, Mito T, Takashima S, Onodera K. Growth and development of the brain in Down syndrome. Prog Clin Biol Res 1991;373:133–52.
[26] Alvarez M, Estivill X, de la Luna S. DYRK1A accumulates in splicing speckles through a novel targeting signal and induces speckle disassembly. J Cell Sci 2003;116: 3099–107.
[27] Himpel S, Tegge W, Frank R, Leder S, Joost HG, Becker W. Specificity determinants of substrate recognition by the protein kinase DYRK1A. J Biol Chem 2000;275:2431–8.
[28] Matsuo R, Ochiai W, Nakashima K, Taga T. A new expression cloning strategy for isolation of substrate-specific kinases by using phosphorylation site-specific antibody. J Immunol Methods 2001;247:141–51.
[29] Woods YL, Cohen P, Becker W, Jakes R, Goedert M, Wang X, et al. The kinase DYRK phosphorylates protein-synthesis initiation factor eIF2Bepsilon at Ser539 and the microtubule-associated protein tau at Thr212: potential role for DYRK as a glycogen synthase kinase 3-priming kinase. Biochem J 2001;355:609–15.
[30] Mao J, Maye P, Kogerman P, Tejedor FJ, Toftgard R, Xie W, et al. Regulation of Gli1 transcriptional activity in the nucleus by Dyrk1. J Biol Chem 2002;277:35156–61.
[31] Woods YL, Rena G, Morrice N, Barthel A, Becker W, Guo S, et al. The kinase DYRK1A phosphorylates the transcription factor FKHR at Ser329 in vitro, a novel in vivo phosphorylation site. Biochem J 2001;355:597–607.
[32] de Graaf K, Hekerman P, Spelten O, Herrmann A, Packman LC, Bussow K, et al. Characterization of cyclin L2, a novel cyclin with an arginine/serine-rich domain: phosphorylation by DYRK1A and colocalization with splicing factors. J Biol Chem 2004;279:4612–24.
[33] de Graaf K, Czajkowska H, Rottmann S, Packman LC, Lilischkis R, Luscher B, et al. The protein kinase DYRK1A phosphorylates the splicing factor SF3b1/SAP155 at Thr434, a novel in vivo phosphorylation site. BMC Biochem 2006;7:7.
[34] Chen-Hwang MC, Chen HR, Elzinga M, Hwang YW. Dynamin is a minibrain kinase/dual specificity Yak1-related kinase 1A substrate. J Biol Chem 2002;277:17597–604.
[35] Skurat AV, Dietrich AD. Phosphorylation of Ser640 in muscle glycogen synthase by DYRK family protein kinases. J Biol Chem 2004;279:2490–8.
[36] Lochhead PA, Sibbet G, Morrice N, Cleghon V. Activation-loop autophosphorylation is mediated by a novel transitional intermediate form of DYRKs. Cell 2005; 121:925–36.

[37] Arron JR, Winslow MM, Polleri A, Chang CP, Wu H, Gao X, et al. NFAT dysregulation by increased dosage of DSCR1 and DYRK1A on chromosome 21. Nature 2006;441:595–600.
[38] Gwack Y, Sharma S, Nardone J, Tanasa B, Iuga A, Srikanth S, et al. A genome-wide Drosophila RNAi screen identifies DYRK-family kinases as regulators of NFAT. Nature 2006;441:646–50.
[39] Alvarez M, Altafaj X, Aranda S, de la Luna S. DYRK1A autophosphorylation on serine residue 520 modulates its kinase activity via 14-3-3 binding. Mol Biol Cell 2007;18:1167–78.
[40] Kim D, Won J, Shin DW, Kang J, Kim YJ, Choi SY, et al. Regulation of Dyrk1A kinase activity by 14-3-3. Biochem Biophys Res Commun 2004;323:499–504.
[41] Yang EJ, Ahn YS, Chung KC. Protein kinase Dyrk1 activates cAMP response element-binding protein during neuronal differentiation in hippocampal progenitor cells. J Biol Chem 2001;276:39819–24.
[42] Mercer SE, Ewton DZ, Deng X, Lim S, Mazur TR, Friedman E. Mirk/Dyrk1B mediates survival during the differentiation of C2C12 myoblasts. J Biol Chem 2005;280:25788–801.
[43] Lee K, Deng X, Friedman E. Mirk protein kinase is a mitogen-activated protein kinase substrate that mediates survival of colon cancer cells. Cancer Res 2000;60:3631–7.
[44] Deng X, Ewton DZ, Pawlikowski B, Maimone M, Friedman E. Mirk/dyrk1B is a Rho-induced kinase active in skeletal muscle differentiation. J Biol Chem 2003;278:41347–54.
[45] Lim S, Jin K, Friedman E. Mirk protein kinase is activated by MKK3 and functions as a transcriptional activator of HNF1alpha. J Biol Chem 2002;277:25040–6.
[46] Deng X, Ewton DZ, Mercer SE, Friedman E. Mirk/dyrk1B decreases the nuclear accumulation of class II histone deacetylases during skeletal muscle differentiation. J Biol Chem 2005;280:4894–905.
[47] Zhou BP, Liao Y, Xia W, Spohn B, Lee MH, Hung MC. Cytoplasmic localization of p21Cip1/WAF1 by Akt-induced phosphorylation in HER-2/neu-overexpressing cells. Nat Cell Biol 2001;3:245–52.
[48] Asada M, Yamada T, Ichijo H, Delia D, Miyazono K, Fukumuro K, et al. Apoptosis inhibitory activity of cytoplasmic p21(Cip1/WAF1) in monocytic differentiation. EMBO J 1999;18:1223–34.
[49] Tatebe H, Shimada K, Uzawa S, Morigasaki S, Shiozaki K. Wsh3/Tea4 is a novel cell-end factor essential for bipolar distribution of Tea1 and protects cell polarity under environmental stress in *S. pombe*. Curr Biol 2005;15:1006–15.
[50] Park GH, Choe J, Choo HJ, Park YG, Sohn J, Kim MK. Genome-wide expression profiling of 8-chloroadenosine- and 8-chloro-cAMP-treated human neuroblastoma cells using radioactive human cDNA microarray. Exp Mol Med 2002;34:184–93.
[51] Oda K, Arakawa H, Tanaka T, Matsuda K, Tanikawa C, Mori T, et al. p53AIP1, a potential mediator of p53-dependent apoptosis, and its regulation by Ser-46-phosphorylated p53. Cell 2000;102:849–62.
[52] Okamura S, Arakawa H, Tanaka T, Nakanishi H, Ng CC, Taya Y, et al. p53DINP1, a p53-inducible gene, regulates p53-dependent apoptosis. Mol Cell 2001;8:85–94.
[53] Taira N, Nihira K, Yamaguchi T, Miki Y, Yoshida K. DYRK2 is targeted to the nucleus and controls p53 via Ser46 phosphorylation in the apoptotic response to DNA damage. Mol Cell 2007;25:725–38.
[54] Geiger JN, Knudsen GT, Panek L, Pandit AK, Yoder MD, Lord KA, et al. mDYRK3 kinase is expressed selectively in late erythroid progenitor cells and attenuates colony-forming unit-erythroid development. Blood 2001;97:901–10.
[55] Lord KA, Creasy CL, King AG, King C, Burns BM, Lee JC, et al. REDK, a novel human regulatory erythroid kinase. Blood 2000;95:2838–46.
[56] Canagarajah BJ, Khokhlatchev A, Cobb MH, Goldsmith EJ. Activation mechanism of the MAP kinase ERK2 by dual phosphorylation. Cell 1997;90:859–69.
[57] Sacher F, Moller C, Bone W, Gottwald U, Fritsch M. The expression of the testis-specific Dyrk4 kinase is highly restricted to step 8 spermatids but is not required for male fertility in mice. Mol Cell Endocrinol 2007;267:80–8.
[58] Isono K, Nemoto K, Li Y, Takada Y, Suzuki R, Katsuki M, et al. Overlapping roles for homeodomain-interacting protein kinases hipk1 and hipk2 in the mediation of cell growth in response to morphogenetic and genotoxic signals. Mol Cell Biol 2006;26:2758–71.
[59] Hanks SK, Quinn AM. Protein kinase catalytic domain sequence database: identification of conserved features of primary structure and classification of family members. Methods Enzymol 1991;200:38–62.