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The Doublecortin Gene Family and Disorders of Neuronal Structure

Thomas Frederik Dijkmans*, Leonarda Wilhelmina Antonia van Hooijdonk, Carlos Patrick Fitzsimons and Erno Vreugdenhil

Division of Medical Pharmacology, Leiden/Amsterdam Center for Drug Research and Leiden University Medical Center, 2333 CC, Leiden, The Netherlands

Abstract: The doublecortin gene family is associated with subcortical band heterotopia, lissencephaly, epilepsy, developmental dyslexia and retinitis pigmentosa. At least 11 genes homologous to the doublecortin gene exist in humans and mice. Cellular processes regulated by different members of the doublecortin family involve neuronal migration, neurogenesis and eye receptor development. Underlying mechanisms include regulation of cytoskeletal structure and microtubule-based transport. Through their doublecortin-domains, doublecortin proteins can bind microtubules and regulate microtubule-dependent processes. However, this regulation is complex and involves many interacting proteins. Moreover, different spatiotemporal expression patterns and the generation of splice variants further contribute to this complexity. The doublecortin-like kinase 1 gene in particular, produces splice variants with different protein domains such as doublecortin-domains, a serine, threonine and proline-rich domain and a serine/threonine kinase-domain. Here, we review our current knowledge on the doublecortin gene family with an emphasis on proteins interacting with doublecortin domains and other domains. In addition, to generate new hypotheses for further research, we analyzed the serine, threonine and proline-rich domain for predicted protein interactions.

Keywords: DCX, DCLK, dyslexia, neuronal migration, neurogenesis, retinitis pigmentosa, doublecortin.

1. INTRODUCTION

The original discovery of doublecortin (DCX) in 1998 linked the DCX gene to subcortical band heterotopia (SBH or "double cortex" syndrome) in females and lissencephaly ("smooth brain" syndrome) in males [1]. Mutations in the DCX gene cause abnormal migration of neurons during development and disrupt the layering of the cortex, leading to epilepsy and mental retardation [2]. When DCX was discovered, it also became apparent that at least one other gene homologous to DCX existed in humans [1]. Today, the DCX family represents a gene family that comprises 11 known paralogues in humans and 11 in mice [3]. Conservation of DCX-related genes is high as orthologues are found across vertebrates and invertebrates [3, 4]. In addition to the described forms of abnormal corticogenesis, mutations in members of the DCX gene family are associated with inherited blindness (retinitis pigmentosa 1 (RP1) ; [5]) and dyslexia (doublecortin-domain containing 2 (DCDC2); [6]). The molecular function (or dysfunction) of doublecortin-related genes is mostly understood from the DCX-domain and its ability to bind microtubules [7]. Regulation of microtubule dynamics via this domain appears to provide a molecular basis for modulation of cytoskeletal architecture [8], mitotic spindle formation [4, 9] and microtubule-based transport [10]. In addition to microtubule binding domains, doublecortin-like kinase 1, 2, and 3 (DCLK1, DCLK2 and DCLK3) encode serine/threonine kinase-domains that show substantial homology to Ca^{2+}/calmodulin-dependent (Cam) protein kinases. Molecular actions of these DCX members are less well characterized, however they appear to regulate cyclic AMP (cAMP) signaling [11]. Moreover, DCX, DCLK1 and DCLK2 have serine, threonine and proline (SP)-rich domains that are thought to mediate multiple protein-protein interactions [10, 12-21] . Further complexity of molecular function of members of the DCX family is rendered by specific spatiotemporal expression, alternative splicing and promoter usage [22]. This review aims to summarize the current knowledge on the role of members of the DCX family in health and disease, to discuss similarities and dissimilarities across the DCX family and to generate hypotheses for further research.

*Address correspondence to this author at the LUMC/LACDR, Div. of Medical Pharmacology, Leiden University, Gorlaeus Laboratories, Einsteinweg 55, 2333 CC Leiden, The Netherlands; Tel: +31 71 527 4428; Fax: +31 71 527 4715; E-mail: tdijkmans@gmail.com

2. THE DCX GENE FAMILY

2.1. Members of the DCX Gene Family

The DCX gene family derives its name from the initially discovered gene *doublecortin*, or DCX [1]. Mutations in the X-linked DCX gene result in severe brain malformation, depending on gender being either lissencephaly or subcortical band heterotopia [1]. In search of genes that underlie retinitis pigmentosa, a form of inherited blindness, the retinitis pigmentosa 1 gene (RP1) gene was identified [23]. It became clear that DCX and RP1 were similar in amino acid sequences and the question was raised whether more related genes were to be found. Recently, a study was dedicated to determine the number and degree of conservation of genes similar to DCX [3]. As the human DCX gene consists of one N-terminal and one C-terminal DCX-domain [24], the human and mouse proteome were searched for proteins containing one or two DCX-domains. This produced 22 such proteins, which are schematically illustrated in Fig. (**1**) and listed in Table **1**. Of these 22 proteins, 10 proteins had orthologues in mice and humans (20 proteins in total), but

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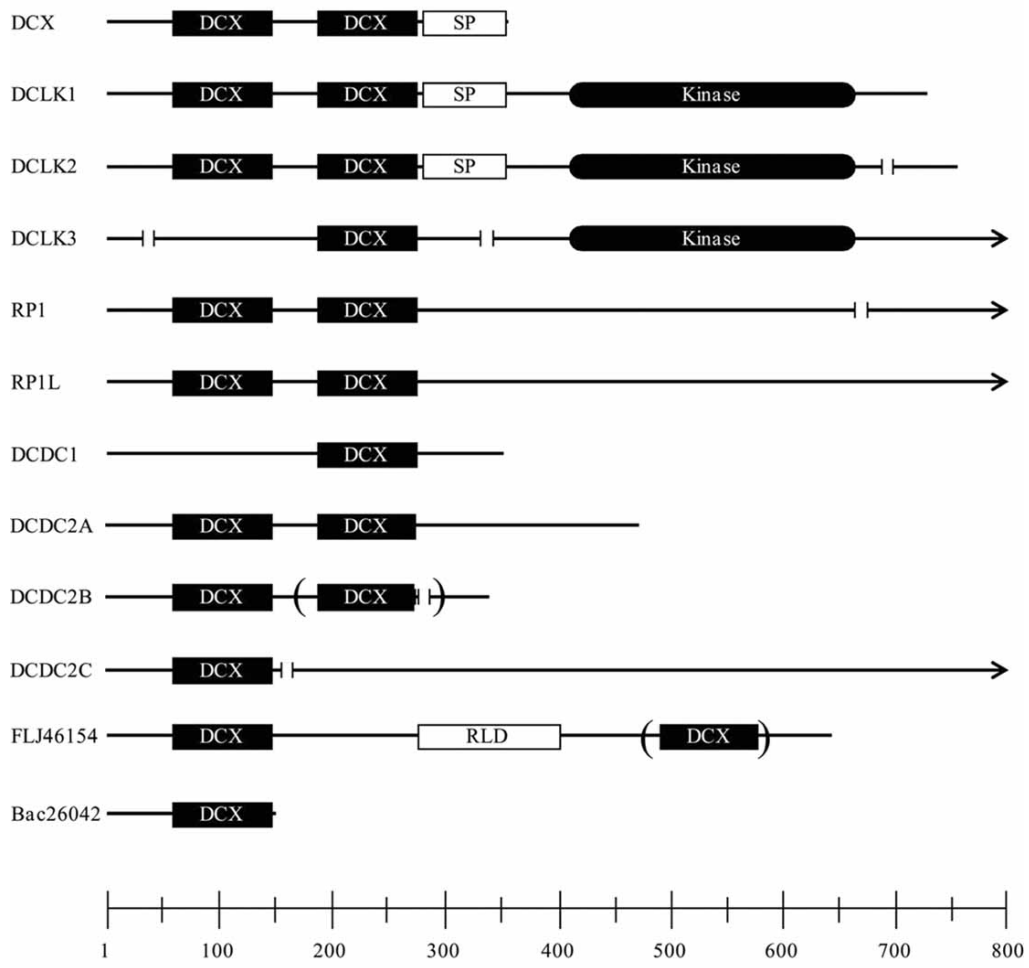

Fig. (1). Overview of the DCX gene family. Schematic representation of proteins produced by the DCX gene family. Protein variants are known for the different genes, however, full-length mouse and human proteins are depicted in order to show all relevant domains. The lower scale bar indicates protein size in number of amino acids. Parentheses enclosing DCX-domains indicate that in the murine orthologue, a DCX-domain has not been found. Two vertical lines (| |) are used to indicate variable proteins lengths to match differences in human and mouse orthologues. Right-pointing arrows indicate protein lengths over 800 amino acids. Positions of DCX-domains have been aligned vertically for clarity, while exact positions within the proteins show more variation. DCX: DCX-domain, SP: serine, threonine and proline-rich domain, RLD: Ricin-type beta-trefoil lectin-domain, Kinase: Kinase-domain.

human DCDC1 (21) had no mouse orthologue and mouse Bac26042 (22) had no human orthologue. Among these proteins, at least three additional conserved domains to the DCX-domain were found. Firstly, a ricin-type beta-trefoil lectin-domain was found in FLJ46154, which may confer binding to carbohydrates [25]. And secondly, a serine/threonine protein kinase-domain was found in both human and mouse proteins DCLK1, DCLK2 and DCLK3. Thirdly, DCX and the DCLK proteins have in between their DCX- and kinase-domain an SP-rich domain, known to be a protein interaction domain [14, 20]. Extension of their search for DCX-related genes revealed that the DCX family is conserved across many species, ranging from unicellular organisms to man [3]. A total of 67 DCX-domain containing sequences were found in humans, chimpanzees, mice, cows, dogs, chickens, fishes, worms, insects, frogs, fungi and sea squirts. Several findings gave information about the evolutionary history of the DCX gene family. For instance, in the majority of the cases, N-terminal DCX-domains were more similar to other N-terminal domains than to C-terminal domains of the same protein. Moreover, the DCX tandem repeat was shown to exist in humans, and already in Dictyostelium. This suggests that DCX-domain duplication is ancient and that the N-terminal and C-terminal have undergone functional specializations [3].

Alternatively, classification of DCLK1, DCLK2 and DCLK3 as evolutionary related proteins was reported by Ohmae and colleagues [11]. Here, instead of searching for genes encoding DCX-domains, a search was performed for kinases with a core catalytic structure similar to Cam Kinases. Their analysis showed that the highest conservation among the three DCLK variants was between DCLK1 and DCLK2 (75.3%). Subsequent comparison showed that DCLK3 is closest to DCLK1 and DCLK2 (56% and 53% identify), followed by Cam Kinase 4 (44% and 42%) and Cam Kinase 2 (43% and 42%). Interestingly, the kinase-

domain of DCLK1-3 has intermediate homology to Cam Kinase 2 and Cam Kinase 1/4, meaning that Cam Kinase 1/4 diverged more from Cam Kinase 2 than from DCLK1-3. In line with Reiner and colleagues [3], DCLK3 was described as less similar to DCLK1 or DCLK2 than DCLK1 and DCLK2 to each other.

Table 1. Protein Lengths of Mouse and Human DCX Family Proteins

	Human	Mouse
DCX	360	365
DCLK1	729	756
DCLK2	695	756
DCLK3	1503	811
RP1	670	2096
RP1L1	2480	1859
DCDC1	348	-
DCDC2A	476	475
DCDC2B	282	339
DCDC2C	1357	214
FLJ46154	648	761
Bac26042	-	148

The invertebrate *C. elegans* also possesses one DCX-domain containing gene, called Zyg-8 [4]. This gene consists of two DCX-domains and a kinase-domain and has a fundamental role in mitotic spindle positioning during the first asymmetric cell division [4]. Zyg-8 may therefore represent an ancestral gene from which the other DCX family genes are derived by gene duplication, deletion and mutation events [26].

2.2. Spatiotemporal Expression of the DCX Gene Family

Besides being homologous in their gene sequences, genes of the DCX family may also share similarities in expression patterns. A correlation analysis between evolutionary relationships and Unigene-derived expression patterns showed that such correlations can indeed be found within the DCX gene family [3]. A first group consisted of the murine and human orthologues of RP1, RP1-like 1 (RP1L1) and DCDC1. Similar expression of these genes was characterized to be high in the eye and low elsewhere in the body. Although in this study [3] DCDC1 was found to be expressed at particularly high levels in the eye, DCDC1 was previously reported to occur at high levels in embryonic brain and testis [27]. Also murine and human FLJ4615 showed expression patterns comparable to this group, with high expression in the eye. However, expression was additionally found in mouse and human liver, and further showed low correlation between human and mouse expression in other tissues. A second group included murine and human DCX, DCLK and DCLK2. The Unigene expression data analyzed in this study [3] showed that for DCX, DCLK1 and DCLK2 particularly high expression can be found in the brain and eye in both mice and man. Other correlations were less evident and additional DCX gene family expression may be found in the liver, kidneys, brain, eyes, muscles, heart and ovaries [3, 11, 28].

2.3. Multiple Genes, Multiple Promoters and Multiple Splice Variants

Alternative splicing and alternative promoter usage by DCLK1 gives rise to transcripts encoding dramatically different protein domains. According to current knowledge, this is not the case for its closest paralogue DCLK2, or other DCX family genes [26, 29]. As this may be important for DCLK1 function, we will briefly review here the known alternative splice variants and classify them according to the presence of different functional protein domains.

The DCLK1 gene produces multiple transcripts from 20 exons [30-32], making use of two alternative promoters and additional splicing routes (see Fig. (**2**) for overview). A first splice variant of the DCLK1 gene is DCLK-short A [30]. This splice variant originates from the 3'-end promoter. DCLK-short encodes the SP-rich domain and the kinase-domain [22, 33]). Two similar splice variants in addition to DCLK-short A (or cpg16 [30, 34]) have been identified. One contains a different C-terminus (DCLK-short B) and a second contains a short 16 amino acid insert (DCLK-short C, [22, 35]). A second type of splice variant of the DCLK gene, called CaMK-related peptide (CARP; also called ania-4) encodes a 56 amino acid long peptide, which largely overlaps with the N-terminal part of DCLK-short and therefore mainly consists of the SP-rich domain [36, 37]. A third splice variant type, called DCLK-long (also called KIAA0369, [38]) is produced from the 5' promoter. DCLK-long is the longest transcript type and therefore contains the most functional domains encoded by the DCLK1 gene. Besides the kinase and SP-rich domain, it contains DCX-domains that enable the protein to bind to microtubules [39]. As for DCLK-short, DCLK-long A, B and C can be distinguished [22, 35, 40]. We and others have cloned yet another splice variant of the DCLK gene, called doublecortin-like (DCL, [9, 41, 42]). This fourth splice variant type is also derived from the 5' promoter. The mouse DCL mRNA encodes a protein of 362 amino acids that shares 73% sequence identity with DCX over its entire length.

3. THE NEUROPATHOLOGIES ASSOCIATED WITH THE DCX GENE FAMILY

3.1. Lissencephaly or Subcortical Band Heterotopia: DCX and DCLK1

The DCX gene is one of the causes of lissencephaly ('smooth brain' syndrome) or subcortical band heterotopia (SBH or 'double cortex' syndrome; [43]). Lissencephaly patients suffer from severe mental retardation and seizures. The cerebral cortex of lissencephaly patients shows clear malformations and lacks the typical laminar architecture and has a smooth outer surface due to the absence of gyri and sulci. SBH patients, who are predominantly women, typically have epilepsy and variable degrees of mental retarda-

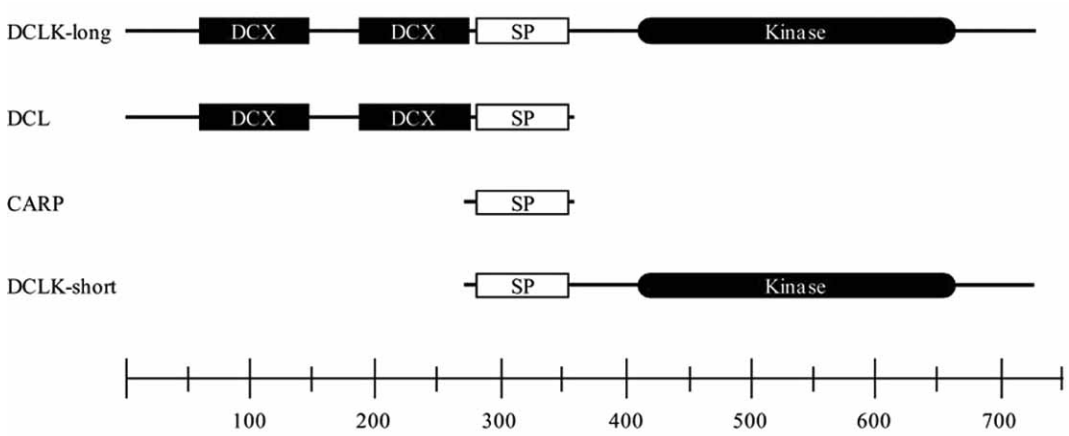

Fig. (2). Splice variants of the DCLK1 gene. Schematic representation of different proteins derived from the DCLK1 gene. Three types of DCLK1 proteins exist in man, rat and human, that consist of combinations of the DCX-domain, the SP-rich domain and the kinase-domain. Sizes are indicative.

tion. SBH is characterized by bilateral bands of heterotopic grey matter between the ventricular wall and the cortex, separated from both [44] . These disorders arise from aberrant neuronal migration during development of the cortex. Therefore, only a brief recapitulation of corticogenesis, necessary for understanding the role of DCX family genes, is given below (for details refer to [45]).

During mammalian embryonic development, cortical neurons are produced in a zone of cellular proliferation lining the cerebral ventricles [46]. Here, cells undergo cell division to create a pool of neuronal progenitor cells. Then, waves of postmitotic neurons are generated, and each successive wave of new neurons migrates from the ventricular zone towards the cerebral surface. Later-born neurons form layers closer to the cerebral surface [47]. At the end of corticogenesis, a six-layered neocortex has developed. The majority of neurons use radial glia cells to travel along, a process called radial migration [45]. Typically, this involves three distinct phases. Firstly, the neuron extends a neurite that bears a leading growth cone. Next, the nucleus is translocated into the neurite (nucleokinesis) and thus along the glial fiber. Finally, the trailing process is retracted, after which these phases are repeated [45].

Clearly, the cytoskeleton is critically involved in these processes, applying to both the actin and the microtubule component of the cytoskeleton [48]. Microtubules provide stability to the extending protrusions, are important in connecting the nucleus to the centrosome (allowing nucleokinesis) and form the basis of the mitotic spindle. Intuitively, microtubule-associated proteins (MAPs) represent important regulators of microtubule dynamics. Various MAPs have also been shown to be important for cortical development, including the *classical* MAPs (the MAP gene family and Tau) and *non-classical* MAPs such as Lissencephaly 1 (LIS1) and DCX.

Remarkably, whereas point mutations in the human DCX gene causes lissencephaly or SBH, DCX-knockout in mouse models revealed little effect on the cortex architecture [49-51]. Only when DCLK1 was knocked out in addition to DCX, a phenotype resulted that resembles the human cortical disorder more closely [52, 53]. Therefore, it appears that functional redundancy between murine DCX and DCLK1 occurs. In support of this, developmental expression of murine DCX and DCLK1 (and DCLK2) seem to be similar [9, 28, 29]. Interestingly, in rat *in utero* RNA interference against DCX only, led to both formation of SBH and laminar displacement. The same RNA interference in mice however failed to induce SBH, but did create laminar displacement [54, 55]. This evidently shows that differences in species are important factors in studying corticogenesis. Indeed, taking into account the highly specialized cortex of man, such differences may be even larger between a rodent and man than between rats and mice [29]. Moreover, the fact that DCX gene deletion and DCX gene knock-down lead to different cortical phenotypes urges caution in interpretations of gene functions.

Although lissencephaly and SBH patients display severe cortical malformations, also hippocampal development is compromised [50, 56]. As a consequence of human DCX mutations, both the granule cell layer and the pyramidal cell layer of the hippocampus are dyslaminated. In contrast to the limited effect of DCX knockout on cortical structure in mice, the hippocampal anatomy is distorted in its cornu ammonis 3 (CA3) region. Moreover, when DCX knockout is combined with DCLK1 knockout, a more severe phenotype is produced, characterized by lamination defects in the entire cornu ammonis and dentate gyrus fields [53, 56]. Apparently, DCX and DCLK1 cooperate during murine hippocampal development, similar to their functional redundance in corticogenesis.

Therapy for lissencephaly or SBH patients remains difficult, although progress is being made. While disrupted neuronal migration in a variety of cortical malformations is considered causal to associated epilepsy; antiepileptic drugs are often ineffective in these cases [57]. Among SBH patients, approximately 65% suffers from refractory epilepsy. Also surgical removal of epileptogenic tissue as an alternative strategy is in general of poor clinical outcome in SBH patients [58]. Recently, however, interesting results have been obtained. Using rat and mouse models, neuronal misplace-

ments as a consequence of manipulated DCX expression were successfully coupled to altered, possibly pathological neurotransmission [51, 59]. Subsequently, correction of neuronal migration through postnatal DCX re-expression was explored in rats [60]. Indeed, cortical malformation could thus be reduced until early postnatal development, and was paralleled by decreased seizure susceptibility. This suggests that therapeutic intervention aimed at (postnatal) DCX activation, either genetically or pharmaceutically, may prove a viable one in the future [60]. Potentially, targeting DCLK1 may also facilitate symptomatic regression in SBH patients.

3.2. Retinitis Pigmentosa: RP1 and RP1L1

The RP1 gene of the DCX gene family is associated with retinitis pigmentosa, which is a type of inherited blindness [23]. Retinitis pigmentosa causes night blindness, progressive loss of the peripheral visual field, bone spicule-like pigmentary deposits and abnormal electroretinograms. The disease ultimately results in severely reduced vision or blindness [23]. Although retinitis pigmentosa is a clinically and genetically heterogeneous disease [61], evidence for a role of RP1 in this retinopathy is growing. The disease of retinal degeneration appears to be caused by photoreceptor death or incorrect outer segment stacking [62]. A brief description of the cellular basis involved is given below, for details refer to [61].

The rod and cone photoreceptors are specialized neurons that lie in the retina and have the same basic structure. From brain towards the visual field, the cell is made up from a synapse, a nucleus, an inner segment, a connecting cilium and an outer segment. The outer segment houses membrane stacks which contain the photopigment that is essential to eye function [63]. These outer segment discs are continuously regenerated by shedding the upper, older discs and producing new discs at the base of the outer segment [64]. Consequently, there is a continuous flow of proteins and other constituents from the inner segment, through the connecting cilium, into the outer segment [63, 65] . The cilium itself is made from microtubules and thus enables microtubule-based transport, mediated by motor proteins kinesin, dynein, myosin and intraflagellar transport proteins [66-68].

The RP1 protein is specifically expressed in photoreceptors and is, by virtue of its DCX-domain, a MAP [69]. Studies with mutant RP1 mice show that wild-type RP1 is required for correct stacking of outer segment discs [70]. Moreover, photoreceptor degeneration, outer segment dysplasia and rhodopsin mislocalization occurs in RP1 disrupted mice [62]. Like RP1, the highly homologous RP1L1 has a DCX-domain, colocalizes to the photoreceptor and is genetically associated with RP [71]. Interestingly, in addition to RP1 and RP1L1, DCLK1 and DCLK2 have been found to be expressed in the mouse photoreceptor sensory cilium complex [72]. Based on the colocalization of these proteins and their shared microtubule-binding properties, it seems likely that they also share a function in outer segment disc morphogenesis.

3.3. Developmental Dyslexia: DCDC2

A third neuropathology associated with the DCX gene family is dyslexia. More specifically, the DCDC2 gene is associated with reading disability or developmental dyslexia [73]. This type of dyslexia is characterized by impairment of reading ability, whereas other intellectual abilities are unaffected. Although the difficulties in learning to read are attributable to specific brain functions, the neurobiological basis is poorly understood [74]. The dyslexic symptoms are complex and suggest complex underlying biological mechanisms. However, 4 gene loci correlate with the occurrence of dyslexia: DYX1CC1, ROBO1, KIAA0319 and DCDC2 [75]. Although the cellular or developmental basis for dyslexia remains unclear, subtle cortical neuronal migration anomalies have been found [76, 77]. This suggests that developmental dyslexia is a disorder of neuronal migration. Indeed, all four genes thus far linked to developmental dyslexia participate in brain development. Two recent studies have experimentally manipulated DCDC2 expression in rat by means of RNA interference and overexpression and studied the cortical consequences. Neuronal migration effects, typical for the dyslexic phenotype [6, 78] were reported.

4. MOLECULAR MECHANISMS OF DCX FAMILY PROTEINS

DCX was identified as a non-classical MAP that may mediate neuronal migration by means of regulating microtubule dynamics [79]. Indeed, regulation of the microtubule cytoskeleton appears to be a major and pivotal substrate of DCX function [80]. With the identification of other DCX family genes that bind microtubules, it seems that regulation of microtubules through a DCX-domain represents a rather conserved molecular mechanism. However, the intricacy of this regulation becomes apparent with an increasing number of studies that report interactions between DCX proteins and other proteins than tubulins. Many of these interactions, although not all, are phosphorylations and dephosphorylations that occur on the SP-rich domain and affect the interaction between DCX and microtubules [12, 14, 15, 17, 19-21] Moreover, the complexity of DCX function seems to reach further than regulation of the microtubule cytoskeleton, as interactions with components of the actin cytoskeleton and transport proteins have also been reported to occur for different DCX family proteins [3, 10, 13, 18, 21, 81, 82]. The SP-rich domain may play an important role here as well. Additional functional complexity applies even more to the members DCLK1-3 of the DCX gene family, which encode a kinase-domain in addition to (partial) DCX-domains and an SP-rich domain. At present, little is known about which proteins these kinases associate with and which mechanisms they regulate.

Here, we will review our current knowledge of protein-protein interactions for the DCX family and discuss the cellular processes involved. Most data comes from the related DCX, DCLK1 and DCLK2 as these genes have so far been studied most intensively. See Table **2** to refer to the interactions described.

4.1. DCX and Microtubule Interaction

The most prominent protein-protein interaction of DCX and the DCLK class is without doubt between the DCX-domain and tubulin. The DCX protein of approximately 360 amino acids in length contains two internal, homologous

Table 2. DCX Family and Associations

Gene Name	Protein Interactions	Associated Cellular Process	Associated Pathology
DCX	Tubulin [79]; Actin [95]; Neurabin II [82]; JIP 1/2 and JNK [14]; Protein phosphatase 1 [19]; Kinesin [14]; AP-1 and AP-2 [13]; Neurofascin [101]; LIS1 [55]; DFFRX [102]	Neuronal migration [79]; intracellular transport [13]	Lissencephaly or laminar heterotopias and epilepsy [115]
DCLK1	Tubulin [39]; Glucocorticoid receptor and Dynein [10]; (JIP 1/2 and JNK [94]); Neurabin II [82]; GRB2 [18, 82]]; Caspase 3 and 8 [16]; Calpain [100]; DFFRX	Neuronal migration ; Retrograde transport [10] ; Neuronal apoptosis [10, 16, 18, 100]; Neurogenesis (cell division) [9, 98]	(Seizures [36, 37])
DCLK2	Tubulin; JIP 1/2; JNK; Neurabin 2; Actin [94]	?	?
DCLK3	Tubulin; JIP 1/2 [94]	?	?
RP1	Tubulin [37]; JIP 1/2 ; (Actin) [94]	Photoreceptor development [116, 117]	Retinitis pigmentosa [116, 117]
RP1L	Tubulin; JIP 1/2; (Actin) [94]	(Photoreceptor development) [118]	(Retinitis pigmentosa) [118]
DCDC1	Tubulin; JIP 1/ 2 [94]	?	?
DCDC2A	Tubulin; JIP 1/ 2 [94]	Neuronal migration [6]	Developmental dyslexia [6]
DCDC2B	Tubulin; JIP 1/2; (JNK); Actin [94]	?	?
DCDC2C	Tubulin; (JIP 1/2) [94]	?	?
FLJ46154	Tubulin; JIP 1/2; JNK; Actin [94]	?	?
BAC26042	Tubulin; JIP 1/2; JNK; Actin [94]	?	?

DCX-domains of approximately 80 amino acids [7]. Although both separate DCX-domains are reported to bind tubulin *in vitro* and *in vivo*, two intact DCX-domains are required and sufficient for microtubule polymerization and stabilization [7]. These microtubule-binding properties are shared with classical neuronal MAPs (MAP2/Tau family, for review see [83]), however, no sequence homology is evident with these functional analogues. Therefore, DCX proteins are considered non-classical MAPs that must have a different biochemical basis for their actions. Indeed, in contrast to the random coil structure of the classical MAPs, DCX-domains adopt a ubiquitin-like fold [84, 85]. Binding of DCX occurs selectively to microtubules consisting of 13 protofilaments and does not affect the growth rate of microtubules [17], but rather acts like an anti-catastrophe factor [86]. Stabilization of these microtubules by DCX is achieved by linking adjacent protofilaments and counteracting their outward bending in depolymerizing microtubules. DCX also enables *de novo* formation of microtubules by microtubule nucleation [86]. Although the conserved C-terminal SP-rich domain is not required for binding of DCX to tubulin *in vivo* and *in vitro*, it is required for specifically binding 13 protofilament microtubules. When bound however, the SP-rich remains accessible for interactions with other proteins [87].

Like DCX, LIS1 was originally identified as a gene which underlies lissencephaly syndrome, when functioning aberrantly. LIS1 is also a MAP and have been reported to function together during corticogenesis [88]. *In vitro* neuronal migration assays showed that interaction of DCX and LIS1 function with dynein to establish nucleus-centrosome coupling [89]. The centrosome is the connecting organelle between the microtubular 'cage', in which the nucleus is enclosed, and the microtubule projection within the leading process of a migrating neuron. When DCX or LIS1 was overexpressed *in vitro* neuronal migration was increased, whereas patient-related mutations in DCX or LIS1 left migration unchanged. Together, it seems that mutations observed in lissencephaly patients can interfere with the interaction between DCX and LIS1. As a consequence, nucleokinesis is disrupted and leads to incorrect neuronal migration [89].

4.2. Regulation of DCX and Microtubule Interaction: PP2, MARK, PKA and CDK5

Interaction between DCX and microtubules is highly dynamic. In particular, it seems that regulation of the phosphorylation state of DCX determines its interaction with cytoskeletal elements and other proteins [90]. This may be inferred from the fact that microtubules within leading processes of migrating neurons are continuous with those in its cell body; however, DCX binds to microtubules preferentially in distal regions [12]. Also, DCX preferentially localizes to microtubules in the distal growth cones of neurites in non-migrating cortical neurons [90]. Therefore, regulators of DCX microtubule affinity must exist that either stimulate distal DCX-microtubule interaction or prevent proximal

DCX-microtubule interaction. Indeed, such regulation occurs through phosphorylation of DCX by kinases (CDK5, PKA and MARK) and phosphatases (PP1 and PP2 [12, 20, 90]).

Seemingly, DCX localization to neurite tips is maintained by phosphatase activity, since treatment of cortical neurons with phosphatase 2 A (PP2A)-inhibitor okadaic acid leads to migration of DCX towards the cell body [90]. In search of kinases that may counteract the phosphorylation state of DCX maintained by PP2A, Microtubule Affinity Regulatory Kinase (MARK) and Protein Kinase A (PKA) were investigated. Both have been reported to use the KIGS-motif - which is present in DCX at amino acid 112-115 (NP_835365) - as a substrate and both are known regulators of MAP function and axon guidance. Although the DCX KIGS-motif appeared not to be the only recognition motif for PKA or MARK, phosphorylation by either kinase reduced co-sedimentation of DCX with microtubules *in vitro* [90]. Mass spectroscopy suggested the additional serine residue 47 of DCX as a common phosphorylation site of PKA and MARK. Interestingly, mutation of S47 in DCX is associated with lissencephaly [7]. Dominant negative mutation of S115A and/or S47A retained microtubule binding, however lost sensitivity to PKA- and MARK-reduced microtubule affinity [90]. Together, these data indicate that dynamic dephosphorylation by PP2A and phosphorylation by PKA and MARK is important for targeting DCX to microtubules in growing neurites.

In a similar fashion, cyclin-dependent kinase 5 (CDK5) phosphorylates DCX thereby affecting neuronal migration [20]. In this study, DCX was shown to occur in a phosphorylated and non-phosphorylated form during development. Based on its amino acid sequence, DCX was predicted to have 9 putative CDK5 phosphorylation sites, which are defined as a Serine-Proline motif surrounded by basic residues. Phosphorylation of DCX occurred through CDK5 *in vitro* and *in vivo* at multiple sites; however, serine 297 was identified as the major site of phosphorylation by means of mass spectroscopy. Using the reaggregate cerebellar granule neuron migration assay [91], overexpression of DCX resulted in enhanced neuronal migration. Subsequent mutation of serine 297 into alanine or aspartic acid created a negative or positive phosphomutant, which both neutralized the increase in migration. Inhibition of CDK5 by roscovitine neutralized the effect of overexpressed DCX, too [20]. These results suggest that phosphorylation of DCX by CDK5, at least on S297 is important for proper neuronal migration. An explanation for the fact that both phosphomutants blocked the migrational increase of overexpressed DCX, is that phosphorylation of DCX must occur according to certain phosphorylation/dephosphorylation dynamics in order to have effect [20].

In search of underlying mechanisms, CDK5 and DCX colocalization was noticed in the perinuclear region and suggested that regulation of nuclear translocation may be responsible for the observed migrational effects. Indeed, phosphorylation of DCX by CDK5 lowered the affinity of DCX for microtubules and inhibited its enhancement of polymerization. Although it is disputed whether serine 297 is the major CDK5 phosphorylation site [15], there is consensus that DCX is a substantial substrate for CDK5 phosphorylation. The fact that CDK5 itself also has extensively been reported to regulate neuronal migration [92] reinforces the notion that CDK5 and DCX are interacting proteins in a pathway shaping neuronal structure and regulating neuronal migration.

4.3. DCX and Microtubule and Actin Interaction: JNK, Neurabin II, PP1, and CDK5

Where phosphorylation may drive DCX away from the microtubule cytoskeleton, it appears that its phosphorylation also increases affinity for the actin cytoskeleton. The c-Jun N-terminal Kinase (JNK) pathway may play an important role in mediating these effects [14]. For all DCX family members, protein interactions have been found to occur with different components of the JNK pathway [14, 93]. The most conserved interaction is between JNK Interacting Protein 1 and 2 (JIP1 and JIP2) and all DCX family members [94]. Additional interaction between JNK 1/2 and DCX and DCLK2 was also shown, and supportive data was found for JNK-DCLK1 interaction [94]. Phosphorylation of DCX by JNK occurs at T321, T331 and S334 [14]. DCX phosphomutants for these residues differentially affected neurite outgrowth of Nerve Growth Factor-stimulated PC12 cells and motility of primary cerebellar neurons, and may therefore be considered functional [14]. In this study, it appeared that JIP connected DCX to kinesin, which can transport the JNK-signaling module to the neurite tip, where JNK then phosphorylates DCX. Interestingly, JNK-phosphorylated DCX was enriched in the actin-rich region of growth cones [14].

In line with this, DCX has been reported to interact with actin proteins, directly and indirectly through Neurabin II [82, 94, 95]. A yeast two-hybrid screen with mouse DCX as bait produced Neurabin II (also called Spinophilin) as an interacting protein – the latter is known as a filamentous-actin (F-actin) binding protein [81]. Tsukada and colleagues [82] demonstrated that introduction of several human DCX mutations in DCX cause the loss of interaction with Neurabin II, and that Neurabin II and DCX are co-expressed in many regions of the developing brain and in primary cultures of hippocampal neurons [82]. Interestingly, when DCX S47E instead of wild type DCX is overexpressed with Neurabin II, the phosphomutant DCX binds not only less to microtubules, but also more to F-actin [95]. Thus, a model emerges in which DCX acts as a cross-linking factor between the microtubule and actin cytoskeleton, that depends on its phosphorylation state and Neurabin II interaction [95].

In addition to this, Neurabin II mediates dephosphorylation of JNK phosphorylation sites on DCX (T326, T336, S339) by protein phosphatase 1 (PP1) in COS-7 cells [21]. When overexpressed, PP1 decreases DCX binding to F-actin and, when inhibited, F-actin binding is retained [21]. Expression of DCX, Neurabin II and PP1 is also found in *in vivo* in migrating neurons [19]. Moreover, PP1, DCX and Neurabin II have been found in a single complex in brain tissue and PP1 has been shown to dephosphorylate the JNK T331 and S334 phosphorylation sites of DCX *in vivo* [19]. Additional functional evidence comes from DCX and Neurabin II knockout mice [12]. Both knockout mice showed abnormal microtubule bundling and similar defects in axon outgrowth. It appeared that Neurabin II facilitates PP1 to dephosphorylate DCX S297 at the 'wrist' of growing axons, whereas

CDK5 phosphorylates this site. Dephosphorylation of DCX by the Neurabin II/PP1-complex at this wrist activates the microtubule polymerizing activity of DCX, required for normal outgrowth [12]. Thus, regulation of phosphorylation and dephosphorylation of DCX is an important mode to govern cytoskeletal organization.

Possibly, similar mechanisms may apply to other DCX gene proteins. In a comparison between all DCX gene members, Neurabin II was found to interact with the most related members DCX, DCLK1 and DCLK2 (but not with other members; [94]). Actin interaction, on the other hand, was observed for DCX and DCLK2 but not for DCLK1. No functional information is available for possible interaction of the JNK pathway and DCLK1 and DCLK2. Interestingly, erroneous eye receptor development as a result of RP1 mutation is associated with reduced JNK signaling [96], suggesting interaction between RP1 and the JNK pathway.

4.4. DCX/DCLK1 and Transport Proteins Interactions

In addition to regulation of cytoskeletal processes within the cell, a number of studies indicate roles for the DCX family in intracellular transport. Firstly, within the SP-rich region of the C-terminal portion of DCX a tetrapeptide YLPL can be found that interacts with the μ1 and μ2 subunits of the adaptor protein (AP) -1 and AP-2 complex [13]. These AP complexes are components of clathrin-coated vesicles. Double labeling and co-immunoprecipitation experiments show that DCX and the AP complexes are expressed in shared subcellular sites of differentiating murine neurons. Also the MAP DCL was found to interact with the μ1 and μ2 subunits of the AP-1 and AP-2 complex [13, 42]. As discussed by Friocourt and colleagues [13] VLDLR and APOE-R2 are receptors that are known to interact with the AP1/2 complex and are important for corticogenesis. Possibly, DCX and DCL regulate import and (microtubular) transport of these receptors [13].

In support of a role in cytoskeletal transport, we have recently shown that DCL mediates glucocorticoid receptor (GR) transport in COS-1, N1E-115 neuroblastoma and rat neuronal progenitor cells [10]. In response to its synthetic ligand dexamethason, the GR translocates from the cytoplasm to the nucleus using the microtubules. It is known that during this microtubular transport, the GR is complexed with heat-shock-protein 90, immunophilins and dynein [10]. Because DCL colocalized with dynein and the GR in neuroblastoma cells, a role for DCL in microtubule-mediated GR transport appeared likely. Indeed, knockdown or overexpression of DCL impaired GR retrograde translocation to the nucleus in neuroblastoma cells and rat hippocampal organotypic slice cultures. Furthermore, co-immunoprecipitation and Förster resonance energy transfer experiments showed that the interaction between DCL and the GR is direct. Interestingly, removal of the N-terminal SP-rich region of DCL retained microtubule colocalization. However, it inhibits GR translocation to the nucleus. Therefore, it appears that trafficking of the GR by DCL occurs through unresolved protein-protein interactions, dependent on its SP-rich C-terminus [10]. Currently, potential suppressive effects of glucocorticoid stress hormones on neurogenesis gain much attention [97]. As DCX family members are implicated in neurogenesis and neuronal migration [9, 28, 53, 98], the DCX family may provide a mechanistic connection between stress hormones and neurogenesis.

Recently, we demonstrated functions of the CARP peptide and DCLK-short, suggestive for additional roles in intracellular transport [18]. First, CARP was found to enhance DCL-induced tubulin polymerization, while CARP alone had no effect. This enhancement is remarkable, because CARP itself has no DCX-domain [18]. In addition, CARP encodes the GKSPSPSPTSPGSLR amino acid motif, which is predicted to associate with growth-receptor bound 2 (GRB2). Typically, this adaptor protein is implicated in retrograde transport of growth factor complexes over microtubules [99]. Co-immunoprecipitation experiments in COS cell protein lysates validated this prediction as recombinant CARP and GRB2 precipitated in a dose-dependent fashion [18]. Remarkably, CARP and DCLK-short appear to play opposing play roles in neuronal apoptosis. CARP was shown to be specifically expressed in apoptotic cells in the dentate gyrus and to exacerbate apoptosis when microinjected in NG108 cells [18]. On the other hand, DCLK-short may be more neuroprotective. The SP-rich domain of DCLK-short contains a PEST-domain, which is subject to cleavage by calpains and caspases during neuronal apoptosis [16, 100]. Where the N-terminal cleavage fragment of DCLK-short - a peptide nearly identical to CARP - exacerbated apoptosis, an uncleavable DCLK mutant protected against neuronal apoptosis [16, 100]. Also DCLK-short contains the GRB2-interaction motif. Thus, DCLK 1 may be involved in growth factor transport and neuronal apoptosis or survival. Indeed, neurotrophic growth factors are well-known regulators of neuronal apoptosis, survival and differentiation [99]. Therefore, it would be of interest to elucidate whether growth factors functions are mediated by DCLK1 or other DCX members.

4.5. Other Interactions

Interaction between DCX and the transmembrane cell adhesion molecule Neurofascin has also been reported [101]. The interaction of DCX with Neurofascin was shown to be dependent on the phosphorylation state of the FIGQ-motif of Neurofascin and may therefore constitute another example of phosphoregulation of DCX function. However, regulation appears complex also here, because the interaction with Neurofascin could not be mapped to an unambiguous fragment of DCX protein and required both DCX-domains. Nevertheless, colocalization of DCX and Neurofascin occurred in migrating neurons and tracts of developing axons. Cell adhesion molecules such as Neurofascin are known to play roles in neuronal migration, neurite outgrowth and fasciculation, synaptic plasticity and axon guidance [101]. Further supporting a role for DCX (and DCL) in cell adhesion and vesicle transport, Friocourt and colleagues [102] showed interaction with the ubiquitin protease DFFRX. Like DCX and DCL, DFFRX associated with microtubules. Interestingly, lissencephaly-causing mutation R192W in DCX, also disrupted interaction with DFFRX [102]. Although the meaning of these two associations with DCX remains so far unclear, it suggests that functionality of DCX may prove broader than currently known.

4.6. DCX/DCLK1-2 and Predicted Interactions Through the SP-Rich Domain

Several data indicate a role for the SP-rich domain of DCX and DCLK1 as an interaction platform for multiple proteins. Ascribed functions so far include cytoskeletal cross-talk and cytoskeleton based transport. To provide basis for further research on this domain, we chose to perform a sequence analysis to identify proteins that may interact with motifs within the SP-rich domain. To increase the likelihood of generating functionally relevant predictions, we were specifically interested in proteins that are predicted to interact with conserved amino acids. For this purpose, we first searched within the human, rat and mouse consensus SP-rich sequence of DCLK1 protein for predicted protein-protein interactions using Scansite 2.0 (http://scansite.mit.edu/ ; [103]). Second, we inspected which of interaction predictions made on this cross-species consensus sequence also apply to the consensus sequence of DCX, DCLK1 and DCLK2 (cross-gene consensus). Fig. (**3A**) schematically depicts the cross-species consensus sequence of 740 amino acids (98% identity, that is 15 aa of 740 not fully conserved) and the associated proteins domains. Fig. (**3B**) shows the surface accessibility as calculated by Scansite 2.0 over the entire length of the consensus DLCK1 protein. As can be seen here, the SP-rich domain has a relatively elevated surface accessibility, which is in line with the notion of the SP-rich domain as a protein docking domain. Fig. (**3C**) is a quantification of the number of predicted protein interactions per amino acid residue. The X-axis of Fig. (**3C**) represents the DCLK1 amino acid sequence, whereas the Y-axis indicates the number of predicted proteins for each particular amino acid. Within the SP-rich region, amino acids 281 until 343 show a pronounced enrichment of 36 predicted interactions (36 of 107 in total). This sequence is given in Fig. (**3D**.) Each underlined amino acid indicates that for that amino acid at least one protein is predicted to interact. As this sequence is the consensus from humans, rats and mice, 2 amino acid positions are substituted by "X". These amino acids were not identical across the three species. To further assess the degree of amino acid conservation to which the predictions apply, we aligned the SP-rich sequences of human DCLK1, DCLK2 and DCX (Fig. (**3E**)). Here, we will discuss predicted interactions with the particular aim of generating new hypotheses for further research on DCX, DCLK1 and DCLK2 function. For reference, we will use amino acid position numbers of the sequence alignment in Fig. (**3E**).

CDK5 was predicted to phosphorylate residue S22, S31, T44, S57, T324 and S64 of DCLK1. Sequence comparison points out that S22 DCLK1 (S298) corresponds to S308 of human DCLK2 (NP_001035350) and to human DCX S297 (NP_835365). This human DCX site was previously reported and discussed as a major CKD5 phosphorylation site, which has an important role in cytoskeletal cross-talk and neuronal migration [20]. In addition to S22, other CDK5 phosphorylation sites were previously predicted and validated [15]. Also our analysis predicts in total 5 phosphorylation sites and supports the validity of predicted interactions. Interestingly, all these sites are highly conserved, as can be seen in Fig. (**3E**). Now that it becomes clear that DCX, DCLK1 and DCLK2 may play redundant functional roles, it seems likely that DCLK1 and DCLK2 are also regulated by CDK5. So far however, no study has assessed these functionalities.

Glycogen synthase kinase 3 (GSK3) is predicted to phosphorylate at S57, S61 and T63. S57 and T63 are highly conserved, whereas S61 shows somewhat less conservation. GSK3 is known to play important roles in neuronal development and has been shown to be an important regulator of microtubule dynamics through phosphorylation of classical MAPs like MAP1b, Tau and MAP2 [104-106]. Moreover, in hippocampal neurons Lis1 and MAP1B have been shown to interact with one another, dependent on GSK3-mediated phosphorylation [107]. As reviewed recently, it is thought that GSK3 helps positioning of the centrosome (or microtubule-organizing center) to achieve cellular polarity during development [108]. Therefore, GSK3 is implied in the same processes as DCX, DCLK1 and DCLK2 are. Together, GSK3-mediated phosphorylation of DCX proteins represents a plausible, yet unknown mechanism to regulate cytoskeletal dynamics.

The highest number of predicted phosphorylations on DCLK1 (and DCLK2 and DCX) apply to mitogen-activated protein kinase (MAPK) extracellular signal-regulated protein kinase 1 (ERK1): S22, T44, S57, S59, S61 and S64. Although phosphorylation by MAPK JNK was previously predicted and validated [14], no study has so far reported phosphorylation by MAPK ERK1 [33]. The many predicted sites, however, suggest that the DCX and DCLK1-2 are likely ERK1 substrates. In support of this, the ERK MAPK cascade plays a crucial role during corticogenesis [109, 110]. The orchestration of cortical development occurs through the actions of growth factors that signal through the MAPK cascade to control cell proliferation, progression and fate decisions of neural progenitor cells [110]. Alteration of MAPK signaling abrogates the generation of a fully populated, normal size cortex [109, 110]. Inhibition of upstream activators or scaffolding proteins of the MAPKs causes neuronal progenitor cells to remain in the subventricular zone in an undifferentiated state. We and others have already described a role for DCL (DCLK1) in determining neuronal fate by regulating mitotic spindle integrity in the proliferative zone [9, 28, 98]. The exact underlying mechanisms remain elusive, however, and are likely to involve phosphoregulation by upstream kinases. ERK1 seems therefore a reasonable candidate to be involved. Moreover, as discussed earlier, interaction with components of the growth factor pathway has already been shown *in vitro* for the DCLK1 protein CARP and growth factor receptor bound 2 (GRB2). In the PC12 cell model, neuronal differentiation induced by Nerve Growth Factor depends on ERK signaling. Moreover, we previously performed a microarray study which revealed that DCLK-short mRNA is among the most strongly induced transcripts during Nerve Growth Factor-induced differentiation [111]. The latter is another indication for a connection between growth factor signaling and DCLK1 function. Given the pivotal role of growth factors in cell fate determination and the emerging role for DCX-related genes in that same process, it would be of interest to test whether growth factor-induced ERK activation projects onto DCX, DCLK1-2 proteins.

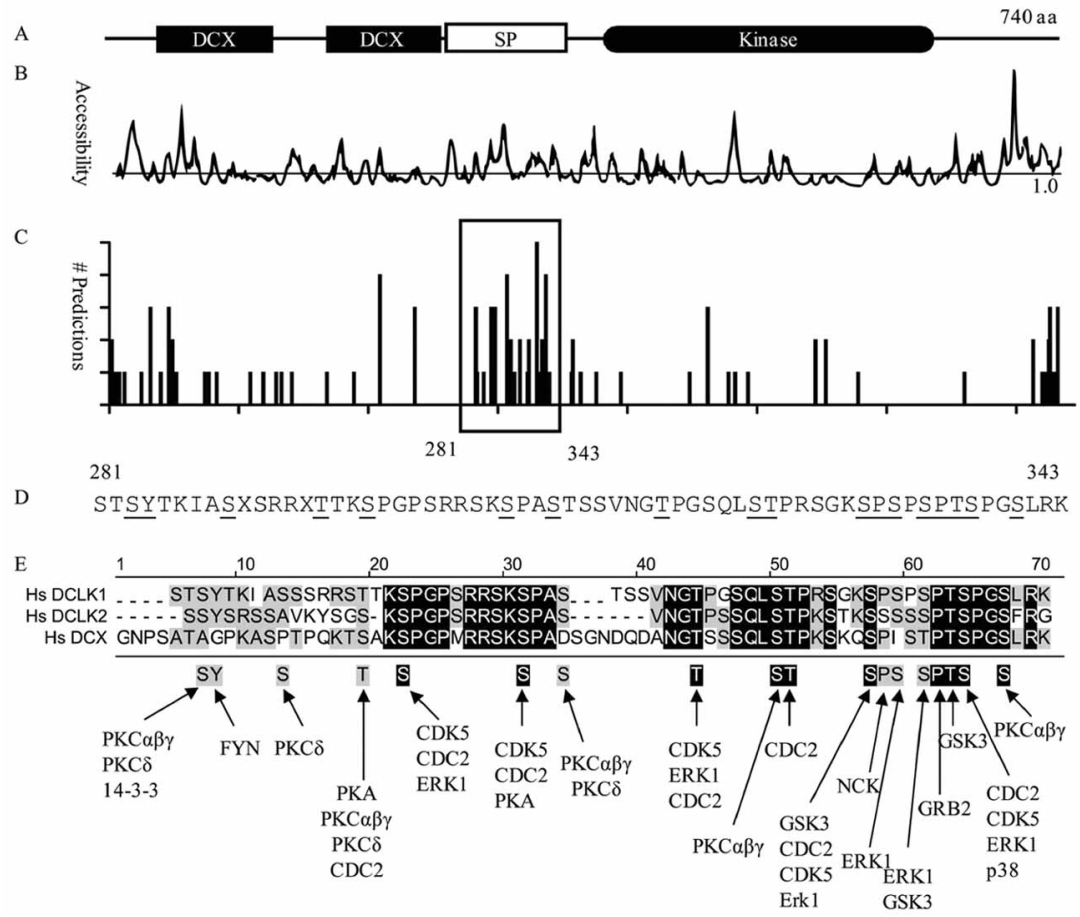

Fig. (3). Predicted protein interactions with the SP-rich domain of DCX, DCLK1 and DCLK2. **(A)**. Visualization based on Scansite analysis from the consensus sequence of human, rat and mouse full length DCLK1 protein sequence (DCLK-long). aa is amino acid. **(B)**. Calculated surface accessibility from the consensus DCLK1 protein sequence (Scansite). X-axis represents the 740 amino acid DCLK1 sequence from N-terminus to C-terminus, whereas the y-axis is an indication of steric availability of DCLK1 subregions. Note the increased accessibility at the SP-rich domain. **(C)**. Quantification of all predicted interactions according to Scansite, which are 107 in total. From N-terminus to C-terminus (740 amino acids), the number of predicted interacting proteins is depicted. The box captures the DCLK1 sequence from aa 281 to 343, which is largely the SP-rich domain and shows a relative high number of 36 predicted interactions when compared across the entire sequence. **(D)**. Consensus sequence from aa 281 to 343. The 2 "X" letters represent two amino acids that are not conserved across human, rat and mouse DCLK1. The underlinings indicate sites that were predicted to interact with at least one other protein (in total 36 predicted interactions). **(E)**. Alignment of the SP-rich sequence of human DCLK1 with human DCLK2 and DCX, together with 36 predicted interacting proteins. Conserved sequence has black background, less conserved grey and different white. The protein interactions predicted from the DCLK1 sequence are visualized underneath the alignment. FYN = Fyn kinase; 14-3-3 = 14-3-3 protein; NCK = NCK adaptor protein 1; α, β, γ and δ indicate kinase subunits. Other protein abbreviations are as specified in main text and abbreviations list.

Finally, protein kinase c (PKC) and cell division cycle 2 kinase (CDC2 or CDK1) are also predicted to substantially phosphorylate DCX, DCLK1 and DCLK2. These kinases are known regulators of microtubule dynamics and provide other interesting candidate proteins to study in relation to DCX protein function [112, 113]. It may be noted that JNK and MARK phosphorylations were not predicted by the present analysis, although known to occur on DCX [90, 93]. Scansite 2.0, however, leaves these motifs unassessed [103].

4.7. DCX Kinases and Substrate Interactions

The function of the kinase-domain within the DCX gene family remains largely elusive, due to limited information on how kinase activity is regulated and on the exact identity of physiological substrates. The kinase-domain is found in proteins from the DCLK1, DCLK2 and DCLK3 gene. As mentioned, DCLK kinases show substantial homology to Cam Kinases 2 and 1/4 [22, 33]. In line with this, the DCLK kinases are Cam Kinase-like serine/threonine kinases that appear to have substrate preferences similar to Cam Kinases. Not only the general kinase substrate myelin basic protein, but also the typical Cam Kinase substrates autocamtide 2 and syntide have been reported to serve as DCLK1 substrates *in vitro* [22, 33, 35, 114]. Using synthetic peptide substrates modeled on synapsin I, a substrate recognition motif for

DCLK1 of Hyd-Arg-X-X-Ser*/Ther*-Hyd was derived [114].

Overexpression of the isoform DCLK1-short (lacking the N-terminal DCX-domain) partially inhibited cAMP-stimulated transcriptional activity of cAMP responsive element binding protein 1 (CREB) [33]. Potentially, this is a more conserved regulatory feature among DCLK kinases: activated DCLK1, 2 and 3, at least by truncation of its auto-inhibitory C-terminal domain, inhibit CREB-mediated gene transcription in a transducer of regulated CREB activity 2 (TORC2)-dependent way [11]. CREB-inhibition is in contrast with classic, activated Cam Kinases, which are known to phosphorylate S133 of CREB and subsequently enhance CREB-mediated gene transcription. Ohmae and colleagues [11] provided support for a model in which TORC2 is phosphorylated by DCLK kinases and leads to CREB response element-inhibition.

In addition to other substrates, it should be noted that DCLK1, 2 and 3 display substantial autophosphorylation [11, 22, 26, 35]. An interesting functional proposition has been made by Edelman and colleagues [26], where they provide *in vitro* experimental support for a connection between autophosphorylation of DCLK2 and microtubule interaction. Enzymatic removal of autophosphorylation groups from DCLK2 greatly increased microtubule affinity, whereas self-phosphorylation seemed to produce DCLK2 protein of lesser affinity. Regulation of microtubule affinity then appears to be the result of dynamic phosphoregulation by the kinase itself and certain phosphatases. In principle, this type of regulation is very similar to the earlier discussed CDK5, JNK, MARK and PKA and phosphatase PP1 and PP2. Provided these *in vitro* findings have physiological manifestations, it raises several interesting questions. First, it would be of interest to identify the autophosphorylation sites of DCLK proteins involved and to see whether these map to the DCX-domain or the SP-rich domain. One autophosphorylation site of DCLK-short has been identified (S382) which is differentially autophosphorylated by DCLK-short A and B [35]. However, functional consequences are unknown. Second, given the conservation among different DCX members, one may wonder whether, for example, DCLK2 phosphorylates DCX. If so, microtubule affinity of DCX may be reduced by DCLK2 at DCLK2-rich subcellular locations. Moreover, differential DCLK1 splicing may implicate regulation of the kinase (DCLK-short) to substrate (DCL) ratio. If DCLK-short expression is upregulated, phosphorylation of DCL may be increased and, consequently, the microtubule affinity of DCL may be reduced.

Upstream regulatory events of kinase activity of DCLK proteins remain so far unknown. Catalytic activity can be increased by introduction of negative charge at T239 of DCLK1, which is in parallel with the activation loop site for regulation of Cam Kinase 1 by Cam Kinase Kinase [114]. Further activation of DCLK1 by C-terminal truncation of an autohibitory domain results in ~6-fold increase, similar to Cam Kinases [11, 33]. Unlike Cam Kinases, activation of DCLK kinases seem to be calcium and calmodulin-insensitive. It has been reported that only cAMP-raising agents affect DCLK-short kinase activity, however other studies could not reproduce this observation [33, 39, 114].

CONCLUSION

The DCX gene family is currently associated with three neuropathologies in humans. Through their DCX-domains, these proteins bind the microtubule cytoskeleton. The affinity for microtubules and filamentous actin is regulated by dynamic phosphorylation and dephosphorylation of DCX proteins. In this way, DCX proteins appear to shape the cytoskeleton and regulate dependent cellular processes, such as neuronal migration, neurogenesis (cell division) and intracellular transport. Although an elegant and seemingly effective mechanism to regulate cellular processes, several important aspects require to be resolved. What are all the cellular processes that DCX proteins are involved in? Do the different DCX proteins regulate different cellular processes? Which molecular mechanisms are the DCX proteins involved in? What are the functions of the different protein domains? To address these aspects, it is paramount to identify and study interacting proteins. We suggest interaction between the SP-rich domain of DCX and DCLK1-2 with several protein kinases including CDK5, GSK3, PKC, CDC2 (CDK1) and ERK. The shown relevance of protein kinases in regulating DCX function warrants future efforts in verifying these interactions.

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LIST OF ABBREVIATIONS

AP	=	Adaptor protein
Cam Kinase	=	Ca2+/calmodulin-dependent protein kinases
cAMP	=	Cyclic AMP or 3'-5'-cyclic adenosine monophosphate
CARP	=	CaMK-like related peptide
CDC2	=	Cell division cycle 2 (CDK1)
CDK	=	Cyclin-dependent kinase
CREB	=	cAMP responsive element binding protein 1
DCDC	=	Doublecortin-domain containing
DCL	=	Doublecortin-like
DCLK	=	Doublecortin-like kinase
DCX	=	Doublecortin
ERK	=	Extracellular signal-regulated protein kinase
F-actin	=	Filamentous actin
GR	=	Glucocorticoid receptor
GRB2	=	Growth-receptor bound 2
GSK3	=	Glycogen synthase kinase 3
JIP	=	JNK Interacting Protein

JNK	=	c-Jun N-terminal Kinase
LIS1	=	Lissencephaly 1
MAP	=	Microtubule-associated protein
MAPK	=	Mitogen-activated protein kinase
MARK	=	Microtubule Affinity Regulatory Kinase
PKA	=	Protein Kinase A
PP	=	Protein phosphatase
RP	=	Retinitis pigmentosa
RP1L1	=	RP1-like 1
SBH	=	Subcortical band heterotopia
SP-rich	=	Serine, threonine and proline-rich
TORC2	=	Transducer of regulated CREB activity 2

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