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Ndel1 alters its conformation by sequestering cAMP-specific phosphodiesterase-4D3 (PDE4D3) in a manner that is dynamically regulated through Protein Kinase A (PKA)

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ABSTRACT

The involvement of the Nuclear distribution element-like (Ndel1; Nudel) protein in the recruitment of the dynein complex is critical for neurodevelopment and potentially important for neuronal disease states. The PDE4 family of phosphodiesterases specifically degrades cAMP, an important second messenger implicated in learning and memory functions. Here we show for the first time that Ndel1 can interact directly with PDE4 family members and that the interaction of Ndel1 with the PDE4D3 isoform is uniquely disrupted by elevation of intracellular cAMP levels. While all long PDE4 isoforms are subject to stimulatory PKA phosphorylation within their conserved regulatory UCR1 domain, specificity for release of PDE4D3 is conferred due to the PKA-dependent phosphorylation of Ser13 within the isoform-specific, unique amino-terminal domain of PDE4D3. Scanning peptide array analyses identify a common region on Ndel1 for PDE4 binding and an additional region that is unique to PDE4D3. The common site lies within the stutter region that links the second coiled-coil region to the unstable third coiled-coil regions of Ndel1. The additional binding region unique to PDE4D3 penetrates into the start of the third coiled-coil region that can undergo tail-to-tail interactions between Ndel1 dimers to form a 4 helix bundle. We demonstrate Ndel1 self-interaction in living cells using a BRET approach with luciferase- and GFP-tagged forms of Ndel1. BRET assessed Ndel1–Ndel1 self-interaction is amplified through the binding of PDE4 isoforms. For PDE4D3 this effect is ablated upon elevation of intracellular cAMP due to PKA-mediated phosphorylation at Ser13, while the potentiating effects of PDE4B1 and PDE4D5 are resistant to cAMP elevation. PDE4D long isoforms and Ndel1 show a similar sub-cellular distribution in hippocampus and cortex and locate to post-synaptic densities. We show that Ndel1 sequesters EPAC, but not PKA, in order to form a cAMP signalling complex. We propose that a key function of the Ndel1 signalling scaffold is to signal through cAMP by sequestering EPAC, whose activity may thus be specifically regulated by sequestered PDE4 that also stabilizes Ndel1–Ndel1 self-interaction. In the case of PDE4D3, its association with Ndel1 is dynamically regulated by PKA input through its ability to phosphorylate Ser13 in the unique N-terminal region of this isoform, triggering the specific release of PDE4D3 from Ndel1 when cAMP levels are elevated. We propose that Ser13 may act as a redistribution trigger in PDE4D3, allowing it to dynamically re-shape cAMP gradients in distinct intracellular locales upon its phosphorylation by PKA.

1. Introduction

Ndel1 (Nuclear distribution element-like; Nudel) is a protein that complexes with cytoplasmic dynein and lissencephaly 1 (Lis1) during mitosis [1,2]; localising to centrosomes during interphase and re-distributing to mitotic spindles during M-phase of the cell cycle [3]. Ndel1 is also critical for regulating the dynein motor complex during nuclear translocation, neuronal migration and in cortical neuronal positioning [4–8]. Furthermore, the loss of the *ndel1* gene results in early embryonic lethality [9], suggesting that it may provide an extremely important functional role during neurodevelopment and within the adult central nervous system.

In the brain, Ndel1 plays a critical role not least through its ability to interact with Lis1 [1], which is an important factor in the aetiology of the debilitating neuronal disease state lissencephaly [10,11] and *D*isrupted *I*n *S*chizophrenia (DISC1), which is now recognised to play a key role in schizophrenia [12,13]. Recently, Ndel1 expression was found to be reduced in the brains of schizophrenics [14]. These data

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suggest that dynamic protein–protein interactions involving Ndel1 are critical for normal brain development and functioning. Indeed, the interaction of Ndel1 with DISC1 is essential for NGF-induced neurite outgrowth in PC12 cells [7,8] and so has implications for the correct development of neuronal pathways.

cAMP is an important second messenger implicated in various neuronal functions including neurotransmission, cellular differentiation and synaptic plasticity [15,16] and compounds inhibiting the degradation of cAMP through cAMP phosphodiesterase activity have considerable therapeutic potential [17–21]. Indeed, a large superfamily of cAMP hydrolysing phosphodiesterases are expressed in a cell type specific fashion [18,19] and serve to tailor cAMP signalling in cells [22,23]. They do this by virtue of regulating cross-talk with other signalling systems and through being targeted to specific intracellular sites and signalling complexes to generate gradients of cAMP within cells to confer compartmentalised cAMP signalling [22,24,25]. Our appreciation of the sophistication of this has increased dramatically over the past few years due to a number of key observations. One is the generation of genetically encoded cAMP sensors that can be targeted to specific intracellular sites [26–34]. Another is that specific PDE4 isoforms have functional roles conferred by signature motifs that allow them to be specifically sequestered to anchor proteins thereby generating intracellular cAMP gradients with unique functional roles [35–39]. Also the critical role that signalling scaffold proteins acting to tether PKA and EPAC sub-populations provide in interpreting cAMP gradients [40–44].

The recent finding that DISC1 can interact with cAMP phosphodiesterase-4 family (PDE4) isoforms [45–47] has led to the exciting possibility that the precise control of neuronal protein complex assembly and its dynamic modulation through second messenger regulation may have important functional consequences in the brain. Indeed, modulation of cAMP levels within different cell types has been observed in the aetiology of schizophrenia for many years [48–52]. Additionally, the PDE4B gene has been linked to schizophrenia [45,53,54], the PDE4 selective inhibitor, rolipram has been mooted as a potential anti-psychotic agent [55–59] and anti-depressive [21,60].

Here we show for the first time that Ndel1 can interact with isoforms from all four of the PDE4 family of cAMP-specific phosphodiesterases and that the interaction of Ndel1 with cAMP phosphodiesterase-4D3 (PDE4D3) is uniquely regulated in a dynamic fashion. In this, activation of PKA is known to cause the stimulatory phosphorylation of PDE4D3 at the conserved Ser54 found in all PDE4 long isoforms together with phosphorylation of Ser13 located within the unique amino-terminal domain of PDE4D3 [61–64]. However, we show here that phosphorylation by PKA at Ser13 within the unique N-terminal region of PDE4D3 confers isoform-specific dissociation of activated PDE4D3. We propose that such a specific interaction may be important for providing a new level of cAMP-dependent modulation for the correct assembly of protein complexes within the central nervous system where specific PDE4 isoforms can be sequestered by Ndel1 and released in an isoform specific fashion. This observation parallels the cAMP-mediated PDE4 isoform-specific release of PDE4C and PDE4D species, but not PDE4A and PDE4B species, from full-length DISC1 and the cAMP-mediated release of all PDE4 sub-family isoforms from N-terminally truncated DISC1 [47].

2. Methods

2.1. Molecular biology

cDNA encoding myc-tagged Ndel1 and pGEX-Ndel1 [2] were kind gifts from Dr Li-Huei Tsai (Department of Pathology, Harvard Medical School, 200 Longwood Avenue, Boston, Massachusetts 02115, USA). The expression and purification of a GST-tagged Ndel1 construct was carried out as described previously by us for other species [65,66]. The cDNA for N-terminally hexahistidine-tagged Ndel1 was generated by PCR using GST-tagged Ndel1 as a template, with the addition of NdeI (5′) and BamH1 (3′) to facilitate cloning into pET28c bacterial expression vector (Novagen) with the oligonucleotide primers 5′-GAT CTG GTT CGC GTC ATA TGG ATG GTG AAG ATA TA-3′ (fwd) and 5′-CAG TCA GTC ACG AGG ATC CTC ACA CAC TGA GAG GCA G-3′ (rev). This was expressed in BL-21 *E. coli* bacterial strain and purified with a Ni-NTA kit according to manufacturer's instructions (Qiagen).

Plasmids encoding PKA inhibitory peptide, PKI and PDE4 constructs PDE4A5, PDE4B1, PDE4C2, PDE4D3, PDE4D5, PDE4D3-S54A, PDE4D3-S54D, PDE4D3-S13A PDE4D3-S13D and D15-PDE4D3 have been described previously [61,67]. The subcloning of the open reading frames of PDE4A4, PDE4B1, PDE4C2 and PDE4D3 into pMALC2 vector (New England Biolabs) to generate N-terminal maltose binding protein (MBP) fusion proteins has been described previously [47].

Oligonucleotides for cloning were purchased from Thermo Electron GmbH, Germany. *Renilla* luciferase (RLuc) fusion protein expression vector (pRLuc(h)-N1) and green fluorescent protein (GFP^2) vector ($pGFP^2$-mcs-Rluc (h)) were purchased from PerkinElmer Life and Analytical Sciences, Boston, USA. The cDNA for N-terminally GFP^2-tagged Ndel1 was generated by PCR using GST-tagged Ndel1 as a template, with the addition of BglII (5′) and ApaI (3′) to facilitate cloning into $pGFP^2$-mcs-Rluc (h) (PerkinElmer) with the oligonucleotide primers:

5′-TCTGGTTCGCGAAGATCTGATGGTGAAGATA-3′ (fwd) and 5′-GTCAGTCACGAGGGCCCCTACACACTGAGAGG-3′ (rev). The 3′ oligo retained the native stop codon, to prevent read-through into the downstream Rluc. The cDNA for C-terminally Rluc-tagged Ndel1 was generated by PCR using GST-tagged Ndel1 as a template, with the addition of BglII (5′) and ApaI (3′) to facilitate cloning into p-Rluc(h)-N1 (PerkinElmer) with the oligonucleotide primers:

5′-TCTGGTTCGCGAAGATCTGATGGTGAAGATA-3′ (fwd) and 5′-CAGTCACGAAGCGGGCCCCACACTGAGAGGCAG-3′ (rev).

The 3′ oligo removed the native stop codon, to facilitate read-through into the downstream Rluc.

The cDNA for N-terminally GFP^2-tagged PDE4D3 was generated by PCR using GST-tagged PDE4D3 as a template, with the addition of HindIII (5′) and ApaI (3′) to facilitate cloning into $pGFP^2$-mcs-Rluc (h) (PerkinElmer) with the oligonucleotide primers:

5′-CTGGGATCCCCAAGCTTCATGCAGGTGAAT-3′ (fwd) and 5′-ACGATGCGGCCGGGGCCCTTACGTGTCAGG-3′ (rev). The 3′ oligo retained the native stop codon, to prevent read-through into the downstream Rluc.

The cDNA for C-terminally Rluc-tagged PDE4D3 was generated by PCR using GST-tagged PDE4D3 as a template, with the addition of HindIII (5′) and ApaI (3′) to facilitate cloning into p-Rluc(h)-N1 (PerkinElmer) with the oligonucleotide primers:

5′-CTGGGATCCCCAAGCTTCATGCAGGTGAAT-3′ (fwd) and 5′-TGCGGCCGCTCGGGCCCCGTGTCAGGAGAACG-3′ (rev).

The 3′ oligo removed the native stop codon, to facilitate read-through into the downstream Rluc. An HA-tagged EPAC1 expression construct was kindly provided by Prof. Johannes L. Bos (Dept of Physiological Chemistry, University of Utrecht, Utrecht, The Netherlands).

All constructs were verified by sequencing.

2.2. Antisera

Antisera specific for PDE4A, PDE4B, PDE4C and PDE4D have been described previously [62,67], a monoclonal antibody to the myc tag of Ndel1 and an antibody to hexa-histidine were obtained from Upstate Cell Signaling Solutions. Monoclonal anti-MBP, anti-VSV and anti-HA antibodies were from Sigma-Aldrich. Anti-sera against the PKA regulatory subunits (RI/RII) and catalytic subunit were from BD Biosciences.

2.3. Scanning peptide arrays

Peptide array libraries were produced by automatic SPOT synthesis and analysed by probing with purified fusion proteins as described in detail before [47,66,68]. The interaction of spotted peptides with MBP