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Spatio-temporal expression pattern of the NatB complex, Nat5/Mdm20 in the developing mouse brain: Implications for co-operative *versus* non-co-operative actions of Mdm20 and Nat5

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ABSTRACT

The NatB complex, Nat5/Mdm20 acetyltransferase mediates *N*-acetylation to control cell cycle progression and actin dynamics in yeast. As yet, little is known about the expression pattern of Mdm20 and Nat5 in multi-cellular organisms. Here we show that Mdm20 is highly expressed in mouse embryonic brain. At E11.5, Mdm20 was widely expressed in both neural progenitors and early differentiating neurons, whereas Nat5 was expressed in Sox1/3+/Mdm20+ neural progenitors. By E14.5, both Mdm20 and Nat5 were downregulated in most ventricular zone neural progenitors, whereas both proteins were found in differentiating neurons and co-expression was maintained at E18.5 in derivatives of these cells, such as midbrain dopaminergic (DA) neurons and septal neurons. These data suggest that Nat5/Mdm20 complex-mediated acetylation may play a role in the proliferation and differentiation of neural progenitors. Intriguingly, our data also showed that Mdm20 is not always co-expressed with Nat5 in all differentiated neurons, for example deep cerebellar neurons. Moreover, detailed examination of the subcellular localization of Mdm20 and Nat5 in cultured Nat5+/Mdm20+ midbrain DA neurons revealed that Mdm20 is also not necessarily co-localized with Nat5 within neurons. Given that Nat5 is only a known member of Nat family protein that interacts with Mdm20, our data imply that Mdm20 may function either with an unidentified Nat protein partner(s) or possibly in a Nat-independent manner.

Post-translational modification of proteins is critically important in biology and expands both the structural and functional diversity of proteins, contributing to both evolutionary divergence and population diversity (Magalon et al., 2008; Arnesen et al., 2009). Two particular protein modifications, acetylation and phosphorylation, play key roles in a wide range of biological events, including tissue development (Kouzarides, 2000; Choudhary et al., 2009; Sadoul et al., 2010). The sculpting of tissue morphology over time is also dependent on the spatial and temporal control of gene transcription (Dessaud et al., 2007; Ohyama et al., 2008; Pearson et al., 2011), and indeed acetylation and phosphorylation often co-operate to modulate protein activities, which in turn modulate gene transcription (Sims and Reinberg, 2008; Lau and Cheung, 2011). For instance, a lysine acetyltransferase (KAT) – formerly called HAT (histone acetyltransferase) – mediates acetylation of the internal lysine residues of histones which, coupled with histone phosphorylation and methylation, controls the de-repression of polycomb-silenced genes, whose temporally-regulated functions are crucial to development (Lau and Cheung, 2011). Conversely, nuclear-located histone deacetylases (HDACs) mediate the repression of gene transcription by the polycomb complex (Sadoul et al., 2010; Garrick et al., 2008).

Recent studies extended the view that reversible acetyl modification of proteins at internal lysine residues is not limited to events in the nucleus but also occurs in the cytoplasm, controlling important biological processes such as translation, cellular apoptosis, motility, and protein quality control (Creppe et al., 2009; Ctalano et al., 2007; Kim et al., 2006; Sadoul et al., 2010). For instance, αTAT1 is responsible for the acetylation of α-tubulin, whereas HDAC6 functions as a α-tubulin deacetylase exclusively in the cytoplasm (Shida et al., 2010; Hubbert et al., 2002). As a consequence, these modifications regulate microtubule stability, and thus cell motility, independent of both histone metabolism and gene transcription (Hubbert et al., 2002). While to date several hundreds of cytosolic proteins have been found to undergo an acetyl modification, the biological significance of this remains to be elucidated (Kim et al., 2006; Choudhary et al., 2009).

N-terminal acetylation is another enzyme-catalyzed reaction whereby N-terminal residues accept the acetyl group from acetyl-CoA. While it takes a place on approximately 80–90% of

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cytosolic proteins in mammals, less attention has been paid to its function (Ametzazurra et al., 2008; Polevoda and Sherman, 2003; Polevoda et al., 2008, 2009). Emerging evidence suggests that an N-terminal acetylation of nascent polypeptides synthesized on polyribosomes plays a pivotal role in cellular homeostasis (Starheim et al., 2008; Arnesen et al., 2010). Based on *in vitro* studies, this modification has been proposed to influence protein function, stability, and subsequent modifications that include phosphorylation. It has been shown to control cell proliferation and protein quality to prevent the aggregation of abnormal proteins (Starheim et al., 2008; Arnesen et al., 2010). Biochemical studies have also shown that in the nervous system serotonin undergoes N-terminal acetyl modification and subsequent conversion to melatonin, thereby regulating the synthesis and metabolism of serotonin as a feedback system (Issac et al., 1990; Miguez et al., 1997; Sugiura et al., 2003). Nonetheless, due to the shortage of *in vivo* studies, it is still not well understood how N-terminal acetylation controls biological events in tissues.

N-acetyltransferase (Nat) complexes are composed of catalytic and auxiliary subunits, and are responsible for the enzymatic reaction to acetylate cytosolic proteins (Ametzazurra et al., 2008; Polevoda and Sherman, 2003; Polevoda et al., 2008, 2009). In yeast, five *N*-alpha-acetyltransferases (NATs), i.e. NatA-E, have been described as catalytic subunits, depending on the distinct amino acid sequences at N-termini they recognize. NatA, NatB, and NatC are three major *N*-acetyltransferases, whereas the substrates for NatD and E are poorly identified. As auxiliary subunits of three major Nat complexes (i.e. NatA, NatB, and NatC), Nat1p, Mdm20p, Mak31p have been identified, respectively in yeast.

In mammals, the NatA complex is composed of a catalytic subunit, ARD1 (arrested defective 1) and an auxiliary subunit NATH (*N*-acetyltransferase human). In the developing mouse brain, both ARD1 and NATH are highly expressed in proliferating progenitors and their expression is downregulated as they differentiate (Gendron et al., 2000; Sugiura et al., 2003). A recent study also showed that NatA complex ARD1-NAT1 is required for the dendritic arbolization of Purkinje cells in the postnatal cerebellum (Ohkawa et al., 2008). These studies clearly indicate that N-terminal acetyl-modification of proteins plays an important role in both the developing and mature brain.

Mdm20 (a regulator of mitochondrial distribution and morphology) is an auxiliary subunit of the NatB complex, the second major acetyltransferase and binds catalytic subunit Nat3 in yeast (Starheim et al., 2008; Ametzazurra et al., 2008; Polevoda and Sherman, 2003; Polevoda et al., 2009). The yeast NatB complex, Mdm20/Nat3 regulates tropomyosin-actin interactions (Singer and Shaw, 2003). In human cells, the NatB acetyltransferase complex is composed of Mdm20 and Nat5 (the human orthologue of yeast Nat3) and has been shown to be essential for cell cycle progression. (Starheim et al., 2008). Given that mouse Nat5 is the closest Nat family protein to human Nat5 and yeast Nat3, mMdm20 and mNat5 are likely to be the auxiliary and catalytic subunits of a mouse NatB acetyltransferase complex, respectively (Starheim et al., 2008; Ametzazurra et al., 2008; Polevoda and Sherman, 2003; Polevoda et al., 2008).

Although *in vitro* studies have suggested the importance of NatB complexes in fundamental cellular events, namely cell proliferation and cytoskeletal organization, none of the subunit proteins

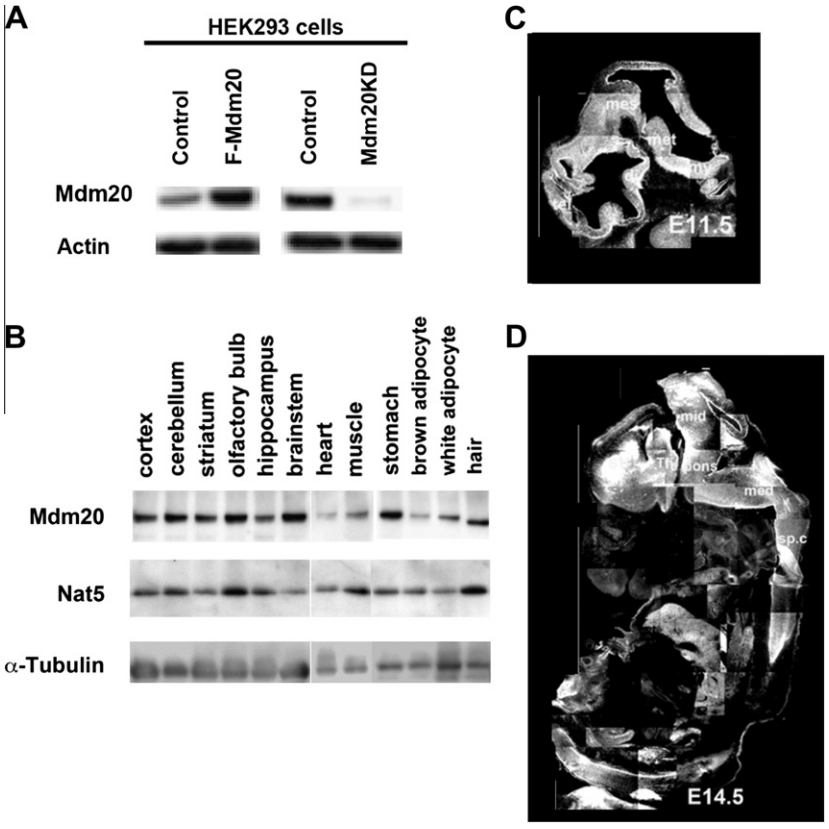

Fig. 1. Mdm20 is highly expressed in the embryonic and adult mouse brains. (A) Western blot of human embryonic kidney (HEK) 293 cells lysates using anti-Mdm20 rabbit polyclonal antibody. When a flag-tagged human Mdm20 was overexpressed (F-Mdm20), an increased expression of Mdm20 was detected. Conversely, an endogenous expression of human Mdm20 was decreased when human Mdm20 was knocked down by human Mdm20 siRNA (Mdm20KD). Western blot of β-actin was used as an internal control. (B) Western blot analysis of adult mouse tissues. Both Mdm20 and Nat5 are highly expressed in the brain (cerebral cortex, cerebellum, striatum, olfactory bulb, hippocampus, brainstem), stomach, and hair, compared to their expression level in heart and muscle. α-Tubulin expression was monitored as internal controls. (C and D) Immunofluorescent labeling of Mdm20 in mouse embryo at E11.5 (C) and E14.5 (D). Sagittal section of the mouse embryonic brain shows that Mdm20 is highly expressed in the brain and spinal cord. tel; telencephalon, mes; mesencephalon, dien; diencephalon, bs; brainstem, str; striatum, sp.c; spinal cord.