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STAM2, a member of the endosome-associated complex ESCRT-0 is highly expressed in neurons

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ABSTRACT

STAM2 (signal transducing adaptor molecule 2), a subunit of the ESCRT-0 complex, is an endosomal protein acting as a regulator of receptor signaling and trafficking. To analyze STAM2 in the nervous system, its gene expression and protein localization in the mouse brain were identified using three methods: mRNA *in situ* hybridization, immunohistochemistry, and *via lacZ* reporter in frame with *Stam2* gene using the gene trap mouse line $Stam2^{Gt1Gaj}$. STAM2 intracellular localization was analyzed by subcellular fractionation and co-immunofluorescence using confocal microscopy. *Stam2* was strongly expressed in the cerebral and cerebellar cortex, hippocampal formation, olfactory bulb, and medial habenula. The majority of STAM2-positive cells co-stained with the neuronal markers. In neurons STAM2 was found in the early endosomes and also in the nucleus. The other members of the ESCRT-0 complex co-localized with STAM2 in the cytoplasm, but they were not present in the nucleus. The newly identified neuron-specific nuclear localization of STAM2, together with its high expression in the brain indicated that STAM2 might have a specific function in the mouse nervous system.

1. Introduction

STAM2 (signal transducing adaptor molecule 2) is a 523 amino acid-long protein encoded by *Stam2* gene that belongs to the STAM protein family. STAM2 has a characteristic domain organization consisting of a VHS domain (Vps-27/Hrs/Stam) at its N-terminus, as well as a UIM (ubiquitin-interacting motif), SH3 (Src homology 3), CC ("coiled coil"), SSM (STAM-specific motif) and C-terminal ITAM (immunoreceptor tyrosine-based activation motif) domain (Endo et al., 2000; Kato et al., 2000; Lohi and Lehto, 2001; Mizuno et al., 2003, 2004).

STAM2 is suggested to play a regulatory role in the endosomal sorting of ubiquitinated membrane proteins. Together with STAM1 and HRS (hepatocyte growth factor-regulated tyrosine kinase substrate) it forms the endosome-associated complex ESCRT-0 (endosomal sorting process required for transport-0) implicated in the sorting of mono-ubiquitinated endosomal cargo. Cargo destined toward the lysosomes for degradation is subjected to the ESCRT machinery, which consists of four protein complexes. A ubiquitinated tag is initially recognized by the ESCRT-0 complex, followed by ESCRT-I, ESCRT-II and ESCRT-III (Hurley, 2010; Roxrud et al., 2010; Williams and Urbé, 2007). A ubiquitin tag can be removed by specific deubiquitinating enzymes: AMSH (associated molecule with the SH3 domain of STAM) and UBPY (ubiquitin-specific protease Y). Both of them bind the SH3 domain of STAM (Kato et al., 2000) and this interaction is involved in endosomal cargo sorting.

ESCRT dysfunction, aberrant endosomal trafficking, and non-proper ubiquitination and deubiquitination are related to many neurodegenerative diseases (Lee and Gao, 2012; Rusten and Simonsen, 2008; Saksena and Emr, 2009; Stuffers et al., 2009). There is increasing evidence that endosomal dysfunction of the most upstream component of the ESCRT system is involved in neuronal cell degeneration (Lee and Gao, 2012). STAM1 and HRS conditional knock-out mice were shown to display a loss of neurons in the CA3 region of the hippocampus (Tamai et al., 2008; Yamada et al., 2001). Knockdown of AMSH or UBPY also impaired deubiquitination in neurons; deletion of AMSH led to neuronal loss in the CA1 subfield of the hippocampus, while the loss of UBPY resulted in embryonic lethality (Ishii et al., 2001; Niendorf et al., 2007). STAM2 knockout mice show no abnormalities at birth, but double knockout of

Abbreviations: STAM, signal transducing adaptor molecule; VHS domain, Vps-27/Hrs/Stam domain; UIM, ubiquitin-interacting motif; SH3, Src homology 3; CC, coiled coil; SSM, STAM-specific motif; ITAM, immunoreceptor tyrosine-based activation motif; HRS, hepatocyte growth factor-regulated tyrosine kinase substrate; ESCRT, endosomal sorting process required for transport; AMSH, associated molecule with the SH3 domain of STAM; UBPY, ubiquitin-specific protease Y; EEA1, early endosome antigen 1.

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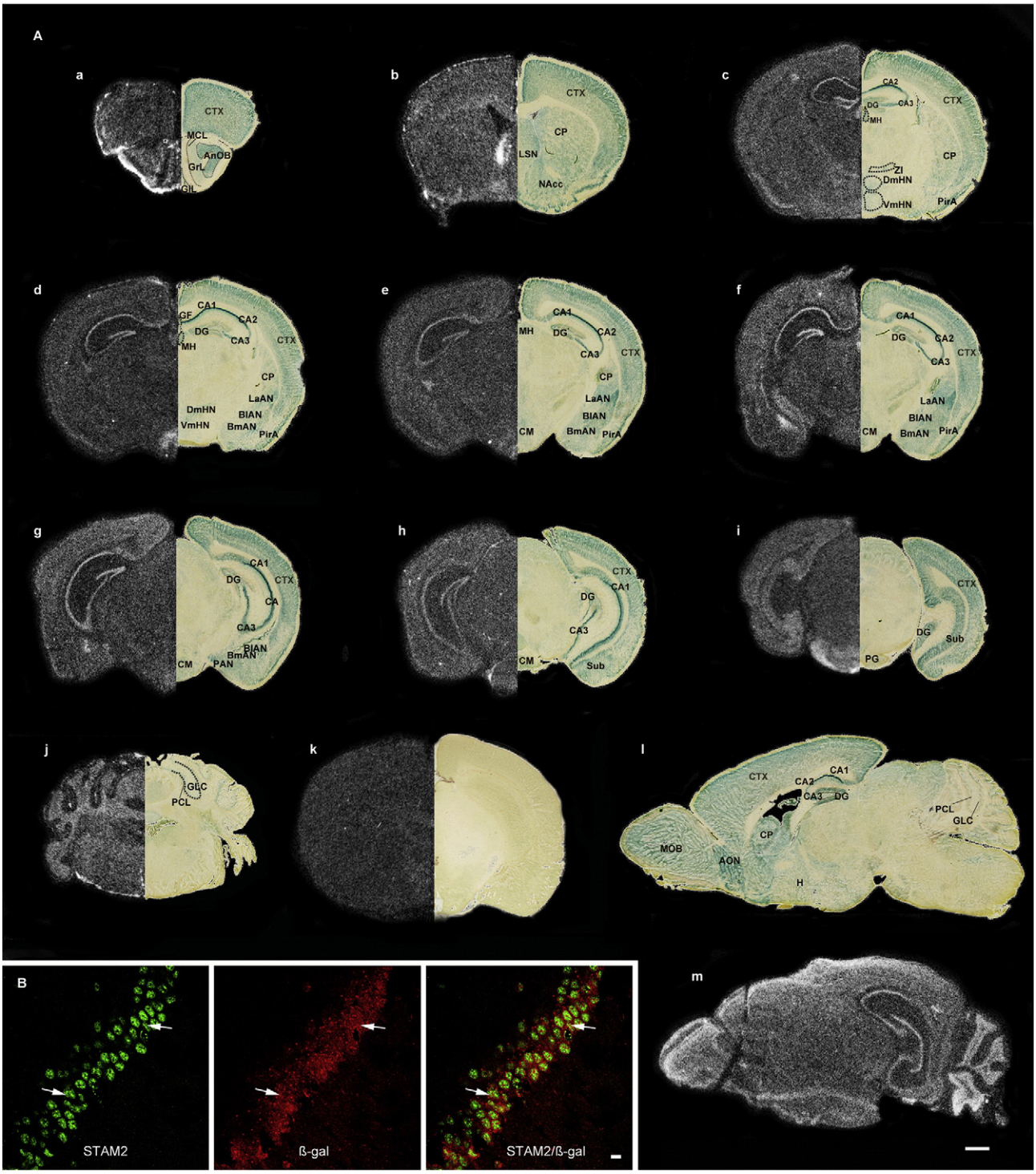

Fig. 1. *Stam2* expression in the adult mouse brain detected by mRNA *in situ* hybridization and β-galactosidase activity. (A a–k) Serial coronal brain cryosections analyzed by mRNA *in situ* hybridization and *lacZ* staining detected by X-gal. The hemisphere on the left represents *in situ* hybridization autoradiographs of 20 μm-thin coronal sections of wild type ($Stam2^{+/+}$) mouse brain, whereas the hemisphere on the right represents β-galactosidase activity staining performed on 50 μm-thin coronal cryosections of $Stam2^{Gt1Gaj/Gt1Gaj}$ mouse brain. (k) Specificity of X-gal staining was confirmed by the absence of the signal in the wild type ($Stam2^{+/+}$) brain sections incubated with X-gal, while specificity of mRNA *in situ* hybridization detection was confirmed by the absence of the hybridization signal in the tissue sections incubated with the *Stam2 sense* probe. (l, m) Representative sagittal section provides an overall view of the distribution of *Stam2* mRNA in wild type (m) and $Stam2^{Gt1Gaj}$ (l) mouse brains detected by *in situ* hybridization (m) and X-gal staining (l). Bar = 1 mm. (B) Confocal photomicrographs showing the co-localization of STAM2 and β-galactosidase in the pyramidal cells of the CA3 region of the hippocampus. Overlap in the distribution of STAM2 and β-galactosidase proteins (arrows) confirmed that *lacZ* insertion in the *Stam2* gene reflected *Stam2* expression. Bar = 10 μm. AnOB — anterior olfactory bulb, BlAN — basolateral nucleus of amygdala, BmAN — basomedial nucleus of amygdala, CA1 — CA1 pyramidal layer of hippocampus, CA2 — CA2 pyramidal layer of hippocampus, CA3 — CA3 pyramidal layer of hippocampus, CM — corpus mamillare, CP — caudoputamen, CTX — neocortex, DG — dentate gyrus, Sub — subiculum, DmHN — dorsomedial hypothalamic nucleus, GF — gyrus fasciolaris, GLC — granular layer of cerebellum, GlL — glomerular layer of the main olfactory bulb, GrL — granular layer of the main olfactory bulb, LaAN — lateral nucleus of amygdala, LSN — lateral septal nucleus, MCL — mitral cells layer of the main olfactory bulb, MH — medial habenula, NAcc — nucleus accumbens, PAN — posterior nucleus of amygdala, PCL — Purkinje cell layer of cerebellum, PGM — pontine gray matter, and VmHN — ventromedial hypothalamic nucleus.