Stam2 expression in the central nervous system during embryodevelopment

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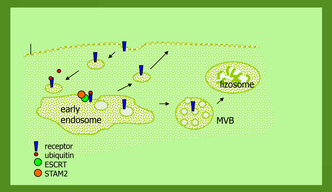
Introduction

STAM2 (signal transduction adaptor molecule 2) is a tyrosine-phosphorylated protein suggested to be involved in cargo selection during endocytic pathway of receptor downregulation, regulation of exocytosis and intracellular signaling.

The aim of the research was to determine *Stam2* expression pattern in the mouse embryos and the brain of newborn mice in order to get insight into its possible role in the embryodevelopment and neurodevelopment.



A mutant mouse line Stam2^{Gt1Gaj} with integration of promotorless *βgeo* (lacZ-neomycin phosphotransferase fusion) gene in *Stam2* gene was used for analysis of its expression by histochemical staining for ß-galactosidase.

Result 1

Sequence determination of the trapped gene in the* Stam2^{Gt1Gaj} *mouse line by 5'RACE and 3'RACE, molecular characterization of vector insertion and mouse genotypization by Southern blotting and PCR (Figure 1).

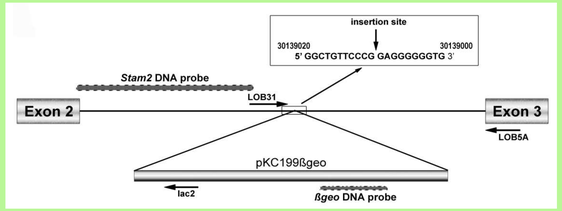


Figure 1. A. Schematic diagram of *ßgeo* insertion into intron sequence of *Stam2* gene between the second and third exon of *Stam2*. DNA probes used for the Southern blot experiments and the primers used for the PCR genotyping were indicated. The insertion site between base 30139010 and 30139009 of the chromosome 2 (Genbank **NT_039206**) is indicated.

Result 2

Stam2 expression pattern determination in mouse embryos by histochemical staining for ß-galactosidase.

The main regions of *Stam2* expression during development are confined to the developing nervous system, heart, lungs, skin, tongue, oral cavity epithelia, intestine, kidney, testis, ovary, submandibular, pituitary and adrenal glands (Figure 2).

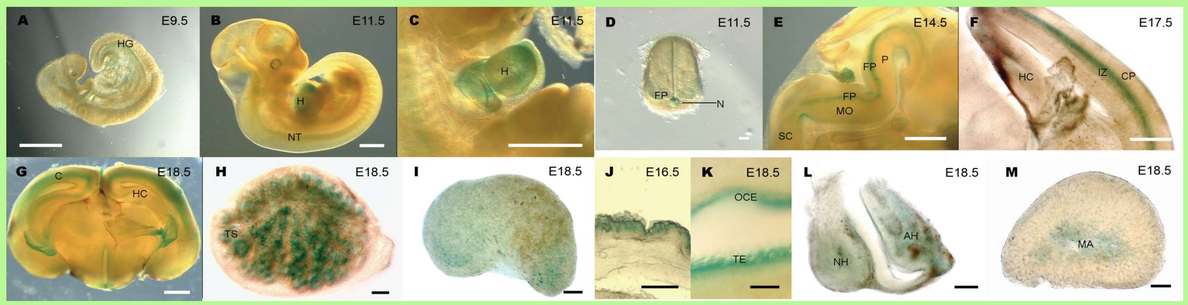


Figure 2. Visualization of ß-galactosidase activity in the representative heterozygous mouse embryos by histochemical staining with X-gal. **A**, **B.** Lateral views of E9.5 and E11.5 embryos, respectively, **C.** the developing heart of E11.5 embryo, **D.** cross section of the neural tube of E11.5 embryo, **E**. sagital section of E14.5 embryo head, **F**. sagital section of E17.5 embryo telencephalon, **G**. transversal section of E18.5 brain, **H**. the testis, and **I**. The ovary at E18.5, **J**. cryo section of the skin at E16.5, **K**. the tongue and oral cavity epithelia at E18.5, **L**. the pituitary, and **M**. the adrenal gland at E18.5. AH – adenohypophysis, C - cortex, CP – cortical plate, FP - floor plate, H - heart, HC - hippocampus, HG - hindgut, IZ – intermediate zone, MA - medulla of adrenal gland, MO - medulla oblongata, NT – neural tube, N - notochord, NH – neurohypophysis, OCE - oral cavity epithelium, P - pons, SC – spinal cord, TE - tongue epithelium, TS - seminiferous tubules. White bars - 1 mm; black bars **-** 0.1 mm

Result 3

3. *Endogenous Stam2 gene expression confirmed by in situ RNA hybridisation and immunohistochemistry.*

X-gal positive regions correspond to strong signals observed by IHC and ISH (Figure 3).

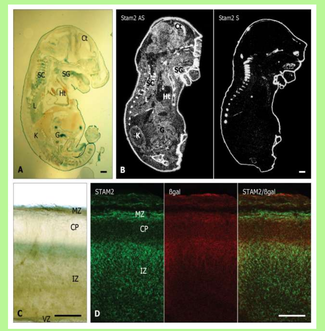


Figure 3. *Stam2* expression in E11.5 and E16.5 embryos detected by *in situ* RNA hybridization and immunohistochemistry confirming the expression pattern obtained by X-gal staining. **A.** ß-galactosidase activity on the cryosection of E16.5 embryo. **B.** *Stam2 in situ* RNA hybridization on E16.5 embryos. Left - antisense (AS) *Stam2* specific probe, right – sense (S) *Stam2* specific probe, as negative control. Bars in **A**. and **B**. – 1 mm. **C.** ß-galactosidase activity on the sagital section of the telencephalic cortex of E16.5 embryo, **D.** STAM2 and ß-galactosidase immunohistochemistry of the sagital section of the telecenphalic cortex of E16.5 embryo. Bars in **C.** and **D. -** 0,1 mm. Ct – neopallial cortex, SC – spinal cord, SG – submandibular gland, Ht – heart, L – lungs, G – gut, K – kidney, MZ – marginal zone, CP – cortical plate, IZ – intermediate zone, VZ – ventricular zone.

Conclusion

The new data on the regions of *Stam2* expression provide additional information for further analyses of the functional roles of STAM2 during development.