Microscopy in the analysis of mouse *Stam2* and *Klf8* genes modified by gene trap procedure

Srećko Gajović, Dinko Mitrečić, Katarina Kapuralin, Nataša Pavlić, and Ljiljana Kostović-Knežević

Croatian Institute for Brain Research, School of Medicine University of Zagreb, Šalata 12, HR-10000 Zagreb, Croatia

srecko.gajovic@hiim.hr
Keywords: brain, embryo, mouse, endosome, gene trap

One of the ways to understand recently obtained hereditary information encoded in the genome is a search for gene function. An insight in the gene function in vivo is enabled by creation of a mouse deficient for the gene of interest. In order to identify genes expressed during central nervous system development and in the same time to create a mutant mouse, the gene trap method was applied.

Embryonic stem (ES) cells were genetically modified by a nonhomologous DNA vector containing a splice acceptor and fused promoterless genes lacZ and neoR. The vector was integrated randomly within the genome, and the inserted genes were active only if the vector was within a transcribed endogenous gene. As a result ES cells were resistant to neomycine (G418) selection. Moreover, expression of lacZ gene mirrored the expression of endogenous gene mutated by gene trap vector, hence its expression could be monitored in ES cells, but as well in ES-cell-derived mutant mice. The insertion was likely to disturb the endogenous gene and an insight in its function could be obtained by analyzes of the phenotype of the mutated mice. Differently from other methods of random mutagenesis, the gene trap vector tagged the mutated genes. This facilitated their identification, which was performed by 5' rapid amplification of cDNA ends (RACE) [1, 2].

The microscopy is crucial in analysis of the expression of the investigated genes and phenotype changes of the mutant mice. Two genes and the corresponding mutant mouse lines were selected for further investigation in our laboratory, *Klf8* and *Stam2*. Klf8 is a transcription factor with unknown function, while Stam2 is a protein involved in cargo sorting along the endocytic pathway.

Expression pattern analyses show both genes to be expressed during brain and heart development (Figures 1 and 2). In the adult brain they are strongly expressed in the cortex and the hippocampus. Visualization of the gene activity was achieved via histochemical assessment of the presence of the beta-galactosidase, and compared to in situ RNA hybridization.

Mouse phenotype analyses include various approaches including morphological analysis, electron microscopy and behavioral analysis. These are expected to reveal through phenotype changes the function of the investigated genes in particular in the developing and in the adult heart and brain.

1. K. Yamamura and K. Araki, Cancer Sci. 99 (2008) p1
2. T. Thomas et al., Transgenic Res. 9 (2000) p395.
3. This research was supported by grants from Unity for Knowledge Fund (No. 35/08), Ministry of Science, Education and Sports Republic of Croatia (No. 108-1081870-1902) and International Center for Genetic Engineering and Biotechnology (No. CRP/CRO06-02).

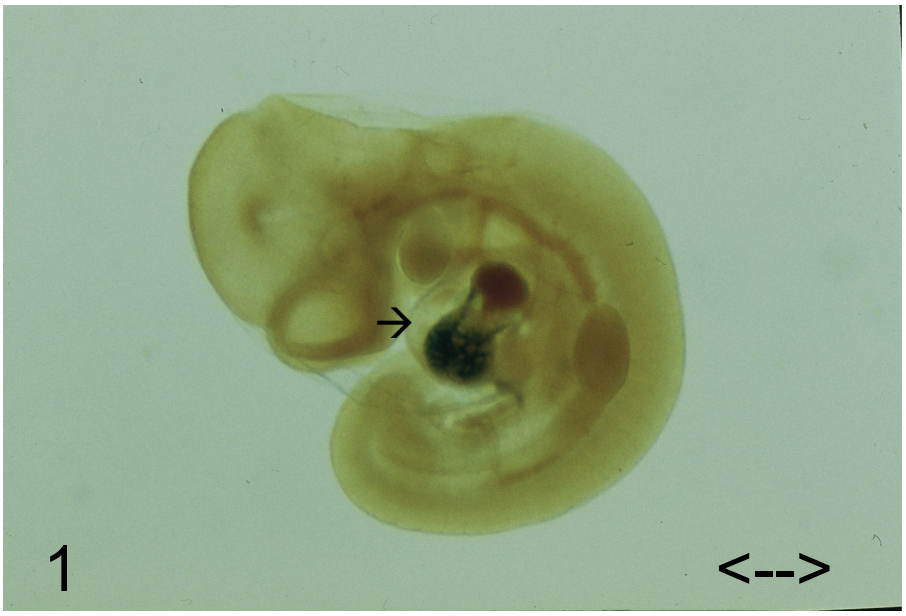

Figure 1. Whole mount staining of 10.5 days old embryo heterozygous for gene trap modification of Klf8 gene. The presence of beta-galactosidase in the heart (arrow) was revealed by use of X-gal. Bar – 1 mm.

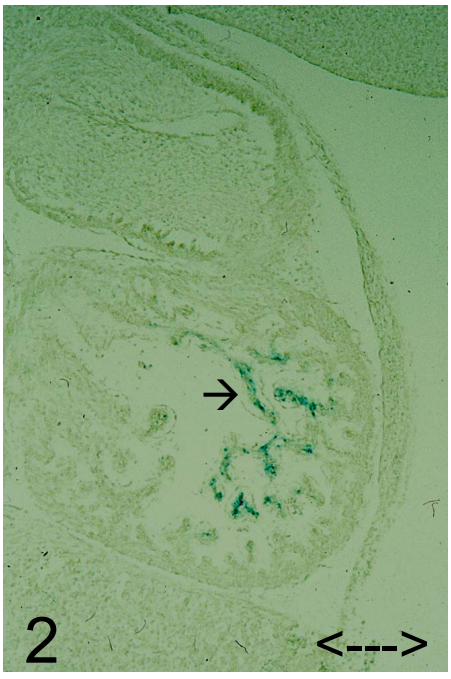

Figure 2. Histological section of the 11.5 days old embryo showing *Klf8* expression in the trabecular system of the heart (arrow). The visualization was done in the same way as presented in the figure 1. Bar – 50 µm

M.A. Pabst, G. Zellnig (Eds.): MC2009, Vol. 2: Life Sciences,
DOI: 10.3217/978-3-85125-062-6-247, © Verlag der TU Graz 2009