# Nucleolar protein 1 (Nol1) expression in the mouse brain

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# Nucleolar Protein 1 (*Nol1*) Expression in the Mouse Brain

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**A B S T R A C T**

*Nucleolar protein 1 (Nol1) is a cell cycle dependent gene highly expressed in proliferating tissues. In order to test whether Nol1 could be used as a marker of dividing neural stem cells within mouse brain, Nol1 expression was analyzed using mouse carrying a gene trap modification of Nol1 gene. High Nol1 expression was found within the hippocampus, olfactory bulb, cerebral and cerebellar cortex. Nol1 was expressed not only in the dividing cells within the brain, but as well in the postmitotic neurons. This suggested a general role of Nol1 in assembling of ribosomes in cells with high protein production.*

***Key words:*** *Nol1, nucleolus, neuron, brain, mouse*

## Introduction

Nucleolar protein 1 (*Nol1*, proliferation-associated nuclear antigen 120, *p120*) is an evolutionary highly conserved gene coding 120-kDa, RNA-binding, nucleolar-specific protein[1,2]. Its expression in the majority of malignant tissues, but also in the non-malignant, highly proliferative tissues correlates with the rapidity of cell cycle[3]. Therefore, *Nol1* was firstly implicated as a tumor cell marker. It is expressed early in G1 phase of the cell cycle and peaks during S phase[1,2]. In clinical praxis *Nol1* expression was shown as a useful prognostic marker of poor tumor prognosis[4,5]. The expression of *Nol1* is induced rapidly following growth stimulation[6], and overexpression of human *Nol1* gene produces tumors in the nude mice[7].

Neurogenesis in mammalian brain is completed at the mature stage[8] and vast majority of neurons escape cell cycle and remain as postmitotic cells. However, some regions of the adult mammalian brain, subventricular zone (SVZ) adjacent to the lateral ventricle, subgranular zone (SGZ) of the hippocampal dentate gyrus, and olfactory bulb[9–12] are able to generate neurons continuously throughout life.

To address the question whether *Nol1* could be used as a marker of dividing neural stem cells within mouse nervous system, mouse carrying a gene trap modification of *Nol1* gene was used. The obtained results, showing high expression of *Nol1* in hippocampus, olfactory bulb, cerebral and cerebellar cortex, suggested that *Nol1* expression in the mouse brain was not specific to the stem cell populations, but mirrored intensive processes of ribosome assembly and high levels of the protein synthesis in the neurons.

## Materials and Methods

### *Nol1$^{gt1Gaj}$ mouse line*

Embryonic stem cell genome was modified by gene trap method. This included the insertion of nonhomologous DNA construct carrying selector (*neoR*) and marker (*lacZ*)[13]. From selected embryonic stem cells, animal carrying the corresponding gene modification was generated. Modified *Nol1* gene was tagged and its expression was monitored by *lacZ* activity[14]. While *Nol1* heterozygotes mate normally, *Nol1* homozygotes are not viable.

### *Tissue preparation and expression analysis*

*Nol1* heterozygotes and their wild type controls were sacrificed and fixed by perfusion of 2% paraformaldehyde and 0.2% glutaraldehyde in phosphate-buffered saline

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(PBS). Brain was removed from the skull and subsequently immersed in the same fixative for one hour. 300 μm thick saggital, frontal and horizontal sections of the brain were obtained using vibratome. Sections were rinsed in PBS and incubated at 37 °C overnight in the staining solution containing X-gal as a substrate for β-galactosidase (0.5 mg/ml X-gal, 2mM $MgCl_2$, 10mM $K_4Fe(CN)_6$, 10mM $K_3Fe(CN)_6$ in PBS). Sections were mounted and photographed using microscope Olympus AX70 and camera Nikon DXM1200.

## Results

In order to analyze expression of *Nol1* gene in the central nervous system of adult mouse, the brains of *Nol1*$^{gt1Gaj}$ adult mice and their wild type controls were cut in saggital, frontal and horizontal directions. The activity of beta-galactosidase was detected by histochemical staining with X-gal, which mirrored *Nol1* expression. In the brain, *Nol1* expression was found within hippocampal and olfactory region and cerebral and cerebellar cortex.

Morphological analysis of all described *Nol1* expressing regions using higher magnifications showed that the signal was visible within nucleoli of *lacZ* positive cells (Figure 1a). This confirmed that beta-galactosidase distribution corresponded to that of *Nol1*, and allowed to distinguish the expressing cells from the rarely observed background, which was represented as diffuse blue staining. In addition, the staining enabled to recognize the typical morphologic features of neurons, and therefore to identify cells expressing *Nol1* as neurons (Figure 1a).

In the hippocampus, expression of *Nol1* was present in the CA1, CA2 and CA3 of Ammons's horn and in the dentate gyrus (Figure 1b and 1d). As *Nol1* expression was confined to the cell nucleolus, *Nol1* presence within Ammon's horn could be assigned to the pyramidal cells of that region. Moreover, *Nol1* was strongly expressed by the granular cells of the dentate gyrus.

Another region highly expressing *Nol1* was cerebral cortex. Single scattered positive cells were visible in all layers, but the main areas of the expression were the external granular layer and the pyramidal layers (layers 2 and 3) (Figure 1c). The presence of staining was confined to the granular and pyramidal cells in those regions. Although expression was found along the whole cortex, the strongest expression was present within temporal caudal lobe in the entorhinal cortex.

Analysis of *Nol1* expression within olfactory bulb revealed *Nol1* expression in all regions where neuronal cell bodies were located. Thus, expression was present in the glomerular cell layer containing small periglomerular neurons, mitral cell layer containing much bigger cell bodies and granular cell layer with numerous small cell bodies (Figure 1e).

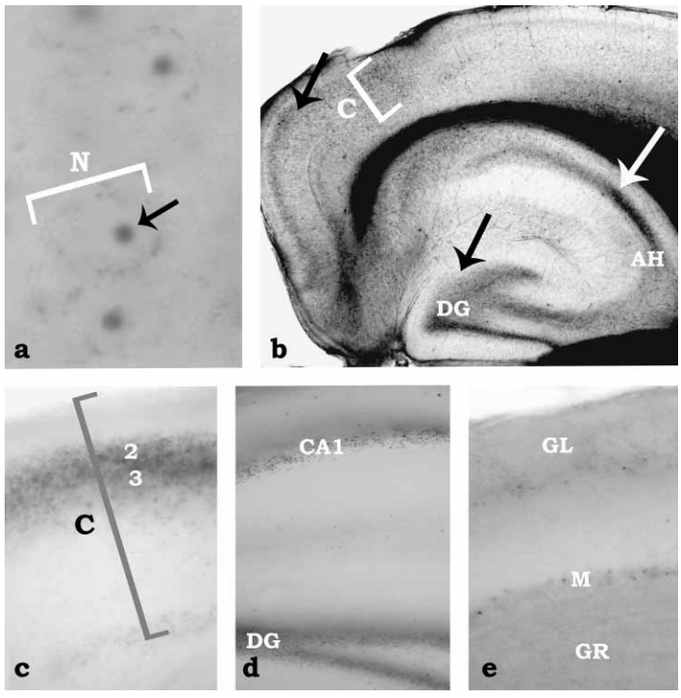


***Fig. 1.*** *Expression pattern of Nol1 within mouse brain. Nol1 expression was localised within a nucleolus of a nerve cell (1a, arrow). The strongest Nol1 expressing regions were cortical layers 2 and 3 (1b, uppermost arrow; 1c) and dentate gyrus (DG, black arrow) and Ammons's horn (AH, white arrow) of hippocampus (1b;1d). Within olfactory region, expression of Nol1 was visible in glomerular (GL), mitral (M) and granular (GR) cell layers (1e). N – nerve cell nucleus, C – cortex, 2 and 3 correspond to cortical layers 2 and 3, CA1 – CA1 region of hippocampus.*

*Nol1* expression in the cerebellum was visible only within granular layer and in Purkinje cell layer, but not in the molecular layer.

## Discussion

The main aim of this work was to verify whether *Nol1* could be used as a marker of proliferating cells within mouse brain, in particular dividing neural stem cells. Following current knowledge about *Nol1* and regions of neurogenesis within rodent nervous system, it was expected that *Nol1* expresson would be found in hippocampus, subventricular zone and olfactory bulb. Although expression in the hippocampus and the olfactory bulb was confirmed, it was present in virtually all cells in these regions. Therefore the fact that *Nol1* was found in the Ammons's horn and the glomerular and the mitral cell layer of the olfactory bulb could not be explained with reports which state that neurogenesis could occur in these regions, as neurogenesis in these regions is of limited extent[12,15]. Fact that *Nol1* was highly expressed in hippocampal, olfactory and cerebral and cerebellar cortex regions, and not in the subventricular zone, suggested that *Nol1* expression in the brain was not specific to the proliferating cells but it was present in the wide population of postmitotic neurons.

Various markers of cellular proliferation in the central nervous system have already been tested[16,17]. Schmetsdorf et al. showed that postmitotic neurons express the cell cycle related proteins previously related only to the mitotic events. Moreover, Migaldi et al.[18] reported presence of *Nol1* in all postmitotic cells 10 years ago. However, high *Nol1* expression in the proliferating cells regularly blurs this fact in the praxis. Our finding of *Nol1* in the postmitotic neurons was in line with these reports. The facts that *Nol1* is cell cycle regulated, expressed in highly proliferative cells, and correlates with tumor grade, led to the widely accepted conclusion that *Nol1* is specific to cellular proliferation. Our finding of *Nol1* in the postmitotic cells implies a more general function of *Nol1* in the process of ribosome assembly. *Nol1* is a mammalian homologue of a yeast protein known as Nop2p, which is required for the methylation of 27S RNA and processing of mature 25S ribosomal RNA (rRNA) during ribosome biogenesis[19]. Mutations in yeast Nop2p protein disrupt rRNA processing by abolishing methylation, therefore suggesting *Nol1* is rRNA methyltransferase. Recent data about factors involved in proliferation and differentiation of the bone marrow cells indicate that these processes require *Nol1*[20]. On the other hand, apoptosis of HeLa cells in the presence of antisense *Nol1* suggests that *Nol1* is required for growth of mammalian cells and therefore the use of antisense *Nol1* in anticancer therapy was proposed[21]. The effects on cellular proliferation are believed to be result of *Nol1* involvement in ribosome assembly. Subcellular localization of *Nol1* within nucleolus supports this hypothesis[22].

Since a cell-cycle dependent function of *Nol1* could not be envisaged in the postmitotic neurons, the additional more general *Nol1* function(s) could be suggested. Location within nucleolus and binding of rRNA indicate the role of *Nol1* in assembling of ribosomes. However, cell cycle dependence should not be strictly tied to the mitotic events, because of several reasons: firstly, genesis of ribosomes, which is extensive in neurons, is in another cell types cell cycle dependent process and this does not necessary implicate that *Nol1* is directly connected to cell cycle events. Moreover, arrest of cell cycle in neurons is not a definite state. Neurons can express cell cycle related proteins in normal[17] or in pathological conditions[23], and neurogenesis can be induced by hypoxia[24]. Although final explanation of *Nol1* expression in postmitotic cells remains elusive, known facts about NOL1 protein and its herein reported presence in the mouse brain, implicate that presence of NOL1 is not only required in the dividing cells, but as well in the cells with high protein synthesis rate, e.g. the brain neurons.

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## IZRAŽAJ BJELANČEVINE JEZGRICE 1 (*Nol1*) U MOZGU MIŠA

### S A Ž E T A K

Bjelančevina jezgrice 1 (Nucleolar protein 1, *Nol1*) je gen izražen u stanicama koje se umnažaju, te mu izražaj ovisi o fazi staničnog ciklusa. Kako bismo testirali hipotezu da je *Nol1* potencijalni biljeg stanica u diobi u mozgu miša (živčane matične stanice), analizirali smo izražaj *Nol1* u mozgu miša kojem je *Nol1* preinačen postupkom genske zamke. Ekspresija *Nol1* je nađena u hipokampusu, njušnom bulbusu, te u kori velikog i malog mozga. Ovaj nalaz je ukazao da *Nol1* nije izražen samo u stanicama koje se dijele, već i u diferenciranim živčanim stanicama. Pretpostavljeno je kako *Nol1* ima ulogu u sintezi ribosoma i bjelančevina u stanicama živčanog sustava.