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Identification of a Mouse Synaptic Glycoprotein Gene in Cultured Neurons*

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Neuronal differentiation and aging are known to involve many genes, which may also be differentially expressed during these developmental processes. From primary cultured cerebral cortical neurons, we have previously identified various differentially expressed gene transcripts from cultured cortical neurons using the technique of arbitrarily primed PCR (RAP-PCR). Among these transcripts, clone 0–2 was found to have high homology to rat and human synaptic glycoprotein. By *in silico* analysis using an EST database and the FACTURA software, the full-length sequence of 0–2 was assembled and the clone was named as mouse synaptic glycoprotein homolog 2 (mSC2). DNA sequencing revealed transcript size of mSC2 being smaller than the human and rat homologs. RT-PCR indicated that mSC2 was expressed differentially at various culture days. The mSC2 gene was located in various tissues with higher expression in brain, lung, and liver. Functions of mSC2 in neurons and other tissues remain elusive and will require more investigation.

KEY WORDS: Primary culture; cerebral cortical neurons; synaptic glycoprotein; bioinformatics.

INTRODUCTION

Neuronal identity, axon projection, and neural circuit formed by synapse networking involve complicated processes, which are intensively studied. In regards to the genetic involvement, more candidate genes that may contribute to neuronal development are being identified (1). Gene isolation and identification could be a laborious task; therefore a simple model with defined functional neuronal differentiation will facilitate the study of genes relevant to neuronal development and differentiation.

We have previously used a system of primary culture of neurons prepared from cerebral cortex of 16-day-old mouse embryos to identify genes that are

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involved in neuronal development (2,3). This type of culture contains an enriched population of GABAergic interneurons (4). We have also shown that neuron-like cells in culture undergo a series of differentiation both in terms of morphology and function. Cells younger than 4 days *in vitro* acquire neuronal morphology but do not show any potassium-induced GABA release, i.e., neurotransmitter release capability, until 7 days *in vitro*. Using the technique of RNA arbitrarily primed polymerase chain reaction (RAP-PCR), we were able to isolate genes, which are differentially expressed in these neurons at various stages of development in culture (2,3). Here we report the identification of a gene, mouse synaptic glycoprotein SC2 (mSC2), using the RAP-PCR approach and publicly available expressed sequence tag (EST) database.

MATERIALS AND METHODS

Primary Culture. Primary cultures of cerebral cortical neurons and astrocytes were prepared as previously described (5–7).

RAP-PCR. Differentially expressed DNA fragments were isolated and identified using RAP-PCR as previously described (2,3).

Northern Blot. Northern blot was performed according to the methods described by Sambrook et al. (8). Total RNA was prepared with TRIzol® reagent (Invitrogen Inc., USA), separated on a 1.0% agarose gel containing 3% formaldehyde, and transferred to a nylon membrane. Blots were pre-hybridized at 42°C overnight in buffer containing 6× SSC, 5× Denhart's reagent, 0.5% SDS, 100 μg/ml denatured, fragmented salmon sperm DNA, and 50% formamide. Hybridization was carried out at 42°C overnight in the same buffer with denatured ^{32}P-dCTP labeled cDNA probe. Hybridized membrane was washed with 1× SSC/0.1% SDS at room temperature and 0.1× SSC/0.2% SDS at 42°C for 30 min 2×, then exposed to X-ray film with an intensifying screen.

DNA Sequence and Gene Assembly. Positive clones were sequenced and then compared with an EST database with FACTURA software (Perkin-Elmer, USA) and with the GenBank database (http://www.ncbi.nlm.nih.gov/blast/). Vector sequences and "trash" sequences, defined as sequences from bacterial sequences and primer polymers, sequences containing >3% of ambiguous bases, and sequences <100 bp long, were removed. The searched results were re-confirmed against the GenBank database for sequence similarity (9).

RT-PCR of the mSC2 Gene. RT-PCR reagents were from Invitrogen Inc., USA and the RT-PCR procedure was as follows: 1 μg total RNA, 0.5 μl RNase inhibitor, and 1.0 μl random primer were mixed at 65°C for 5 min and stored in an ice bath. RNase inhibitor (0.5 μl), 5× first strand buffer (4 μl), 10 mM dNTPs (1 μl), 0.1 M DTT (2 μl), 200 U/μl Superscript II (1 μl), were added and incubated for 1 h at 37°C. The reaction mixture was transferred to 95°C for 5 min, and then stored in an ice bath. The PCR reaction was as follows: RT products (0.2 μl), 25 μM primers (0.5 μl each), 10× PCR buffer (2 μl), 2.5 mM dNTPs (1 μl), *Taq* DNA polymerase (1 μl). The mixture was incubated at 94°C for 5 min, then subjected to 35 cycles of 94°C for 45 s, 55°C for 45 s, 72°C for 1.5 min and a final extension at 72°C for 10 min. The PCR products were analyzed in a 1.0% agarose gel.

RESULTS AND DISCUSSION

In our present study, we have utilized the RAP-PCR technique and identified a few ESTs tentatively exhibiting differential gene expression in cortical neurons at different culture ages. Although RAP-PCR and differential display reverse transcription PCR (dd RT-PCR) (10) share similar principles, RAP-PCR is comparatively simpler to manipulate. RAP-PCR has therefore become more widely used in isolating and cloning differentially expressed genes (11). One of these EST clones, 0–2, exhibited high sequence similarity to human and rat synaptic glycoprotein SC2. In order to obtain full-length sequence of 0–2, an EST database and FACTURA software were utilized to align contigs similar to 0–2 and the full-length 0–2 transcript was named as mouse synaptic glycoprotein (mSC2). The full-length mSC2 transcript contains 1132 bp, slightly shorter than the rat SC2 (1178 bp) and human SC2 (1146 bp, 1111 bp, and 629 bp spliced variants) homologs. Figure 1 shows the parallel comparison of mSC2 with other mammalian SC2.

RT-PCR result has shown that mSC2 is expressed differentially in neurons of various culture age, with the highest expression apparently on day 7 (Fig. 2). The mSC2 transcript is also observed in the cortical tissue of newborn mouse (Fig. 2; rightmost lane). Similar expression pattern can also be revealed by Northern blot (Fig. 3), indicating a highest expression level of mSC2 in 7-day-old cultured neurons. This apparent increased expression of mSC2 could also be observed in mouse cortical tissues of various age (E16 to 14 days) (data not shown), indicating cultured neurons display physiological response (increased mSC2 expression) similar to the *in vivo* conditions. Intriguingly, RT-PCR uncovers the expression of mSC2 in non-neural tissues, such as lungs, kidney and liver, in addition to cortex and cerebellum (Fig. 4). Moreover, there are two visible PCR bands in most non-neural tissues, except testis, indicating a possible presence of at least two splice variants of the mSC2 transcript, like the human mSC2 homolog (9). In fact, the extra PCR band observed in various tissues was subjected to DNA sequencing, and the obtained sequence was compared

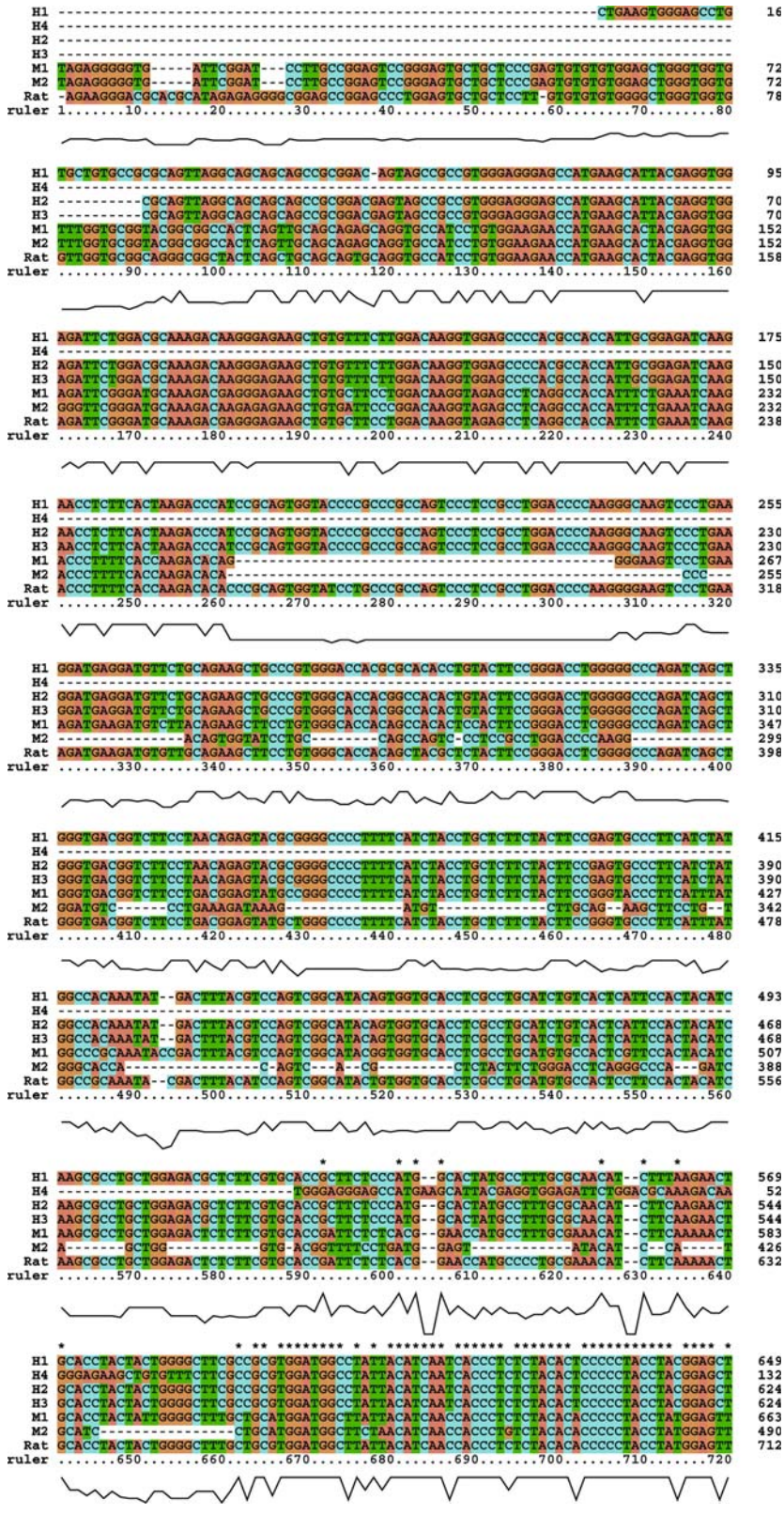

Fig. 1. Continued.

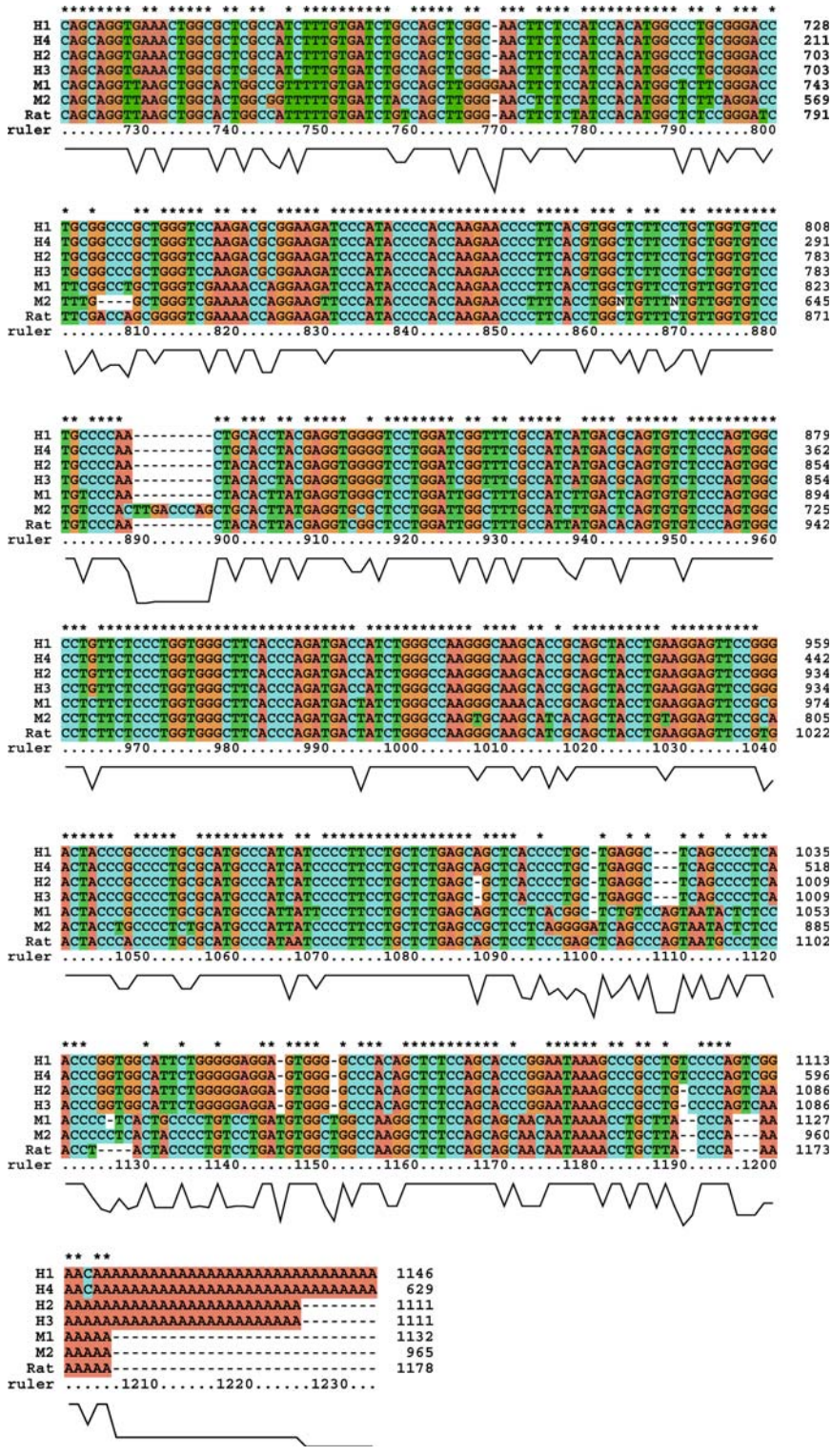

Fig. 1. DNA sequence comparison of the rat, human and mouse SC homologs. H1: human synaptic glycoprotein SC2; H2: human synaptic glycoprotein SC2 variant 2; H3: human synaptic glycoprotein SC2 variant 3; H4: human synaptic glycoprotein SC2 variant 4; M1: mouse synaptic glycoprotein SC2 (mSC2); M2: mouse synaptic glycoprotein SC2 variant 2; Rat: rat synaptic glycoprotein SC2.

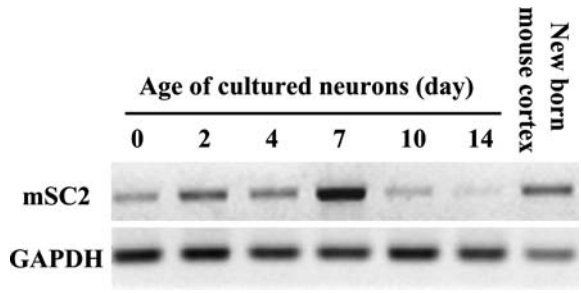

Fig. 2. Expression of mSC2 in cortical neurons at various culture age and in newborn mouse cortex as revealed by RT-PCR. The expression level of mSC2 relative to GAPDH peaks at day 7 and shows a decreasing trend at day 10, and 14. Newborn mouse cortex shows expression of mSC2 as well.

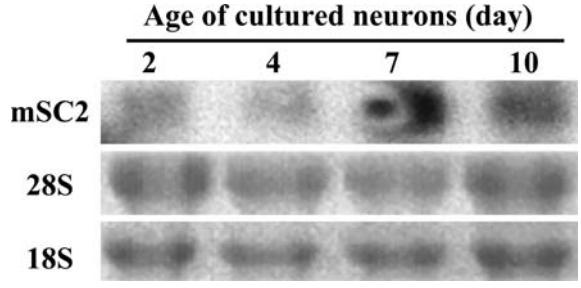

Fig. 3. Expression of mSC2 in cortical neurons at various culture age as revealed by Northern blot. Highest expression level of mSC2 for 7-day-old cultured neuron is consistent with the result indicated by RT-PCR (Fig. 1). The Northern blot experiments were repeated twice.

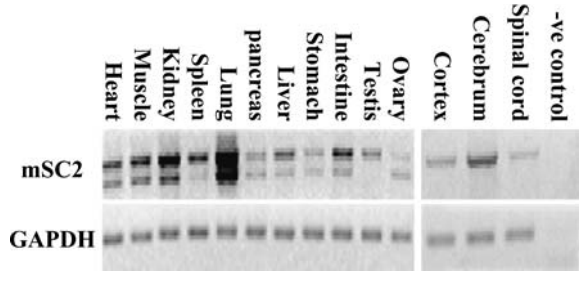

Fig. 4. Tissue distribution of mSC2 in mouse. There are two observed bands (upper band: mSC2; lower band: mSC2-variant 1) for most tested tissues, except testis and neural tissues. Further characterization will be required for the extra PCR band.

with mSC2 and other mammalian SC2 (Fig. 1). This extra variant was thereafter named as mSC2-variant 1. The mSC2 transcripts of various sizes may play different roles in different tissues. Cultured astrocytes and C6 glioma cell line also express mSC2, further

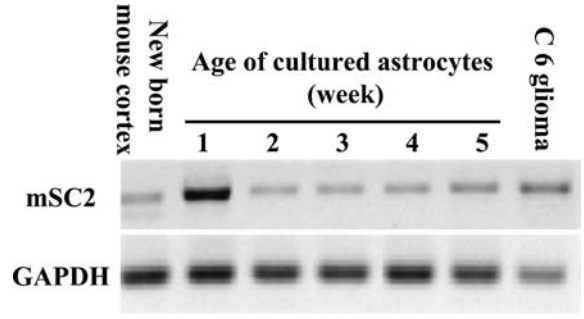

Fig. 5. Expression of mSC2 in cultured astrocytes at various culture age and in C6 glioma. Highest mSC2 expression can be seen for 1-week-old cultured astrocytes, and the mSC2 expression levels remain lower through to 5 weeks. C6 glioma also exhibits expression of mSC2.

strengthening the presence of mSC2 in many different types of tissues (Fig. 5).

Similar to mouse SC2 gene, the rat SC2 gene can be found in neural tissues, including the cerebellum, cerebral hemispheres and brain core, with expression peaking in cerebellum between day 15 and 20 after birth (12). Rat SC2 is present in non-neural tissues, such as pronounced expression of rat SC2 in liver and heart, but trace amount in kidney. Moreover, *in situ* hybridization results show that rat SC2 gene is abundant in neuronal-enriched areas, and sparse in glial-enriched areas (12). These results suggest some roles of SC2 in both neural and non-neural tissues.

Glycoproteins are thought to play a role in the development and/or maintenance of synapses in the mammalian central nervous system (CNS). However, our results suggest that mSC2 and its homologs may be involved not only in development and maintenance of synapses of the CNS, but also in other important cellular functions (13). But the functions of SC2 remain elusive in other tissues since recent reports have indicated participation of SC2 in regulating the development of neural tissues (14–16). This was supported by the finding that SC2 is not a neuronal specific gene, and may function in other cells or tissues. Highly similar genes have also been cloned from a human tumor cell line (GenBank: AK001416) and dermal papilla cells (GenBank: AF222742). For further confirmation of their functions and distribution, one could use antibodies and *in situ* hybridization to detect whether the proteins are being secreted or not, and located in the synapse.

In conclusion, mSC2 is expressed in both neuron and astrocytic cultures. It is also identified in different tissues, such as brain, heart, liver, spleen, kidney and

lung. This indicates that mSC2 may act as an intrinsic cellular factor. Expression of mSC2 is highest in cultured neurons at 7 days, a culture time point at which cultured neurons release neurotransmitter under high potassium treatment (4). This suggested that mSC2 gene might be related to maturation of synaptic function during neuronal development. Moreover, there are at least two transcript sizes for mSC2 in various tissues, similar to the human SC2 homolog, suggesting further that mSC2 may serve different functions in various tissues.

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