ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol Exp 2005, 65: 161-165

The expression and function of Sgt1 protein in eukaryotic cells

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Short communication

Abstract. The Sgt1 protein was discovered in yeast but later it was found in other eucaryotic organisms, including plants and mammals. It was suggested that the Sgt1 protein plays a role in the CBF3 kinetochore and the SCF ubiquitin ligase complexes. Since the distribution, properties and functions of the mammalian Sgt1 have not been extensively studied, we examined the expression of Sgt1 protein in the rat tissues and here we report the preliminary results. Using northern and western blots we found that the Sgt1 protein is expressed in various tissues and that brain, skeletal muscles and spleen contain high levels of this protein and its mRNA. Then, by western blot, we checked the localization of Sgt1 in some areas of the rat brain such as cortex, cerebellum and hippocampus and we found that cerebellum and cortex contain highest amounts of this protein. Using immunohistochemistry we found that the Sgt1 protein is present in both neurons and glial cells.

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Key words: Sgt1 expression, plant, yeast and mammalian cells, target proteins, brain tissue

The Sgt1 gene (suppressor of G2 allele of Skp1) was discovered in yeast cells (*S. cerevisiae*) by Kitagawa and coauthors (1999). It was shown that Sgt1 binds to Skp1 and that it is required for the function of the SCF ubiquitin ligase and the CBF3 kinetochore complexes. The possible function of Sgt1 in the yeast kinetochore complex assembly was further studied by Rodrigo-Brenni and coauthors (2004). The authors proved that the Sgt1–Skp1 interaction was a critical step in assembling Ctf13 and Ndc10 proteins within the CBF3 complex. The proposed role of Sgt1 in kinetochore assembly is in agreement with the results obtained from the immunofluorescence analysis showing that this protein is present not only in the cytoplasm but also in the nucleus (Dubacq et al. 2002).

The Sgt1 protein was later found in multiple plant and mammalian cells (Azavedo et al. 2002, Niikura and Kitagawa 2003, Steensgaard et al. 2004, Yamamoto et al. 2004). In plant, Sgt1, together with its target protein, RAR1, interacts with Hsp90 and that this protein complex modulates activity and stability of the essential components of signaling pathways leading to pathogen resistance (Hubert et al. 2003, Shirazu and Schulze-Lefert 2003, Takahashi et al. 2003). Plant Sgt1 was also found to bind to the ubiquitin-conjugating enzyme, Rad6, and to be involved in auxins' degradation *via* SCF^{TIR} ubiquitin ligase (Gray et al. 2003, Yamamoto et al. 2004).

Recently, expression of Sgt1 in human tissues has been studied. By applying the reverse transcription-polymerase chain reaction and western blot technique it was shown, that Sgt1 is present in all tissues examined (Niikura and Kitagawa 2003, Zou et al. 2004), although its level varies depending on the tissue type. The highest level of Sgt1 was found in the human brain, liver, lungs and testis. Because of a limited accessibility of the human material for further studies concerning the localization and functions of Sgt1, rats were used in our experiments.

All procedures on live animals were approved by the Local Ethics Committee and strictly conform to the Polish and international standards. First, we examined expression of Sgt1 and for that we applied the northern and western blots using the specific cDNA probe and polyclonal antibodies, respectively. Total RNA was isolated according to Chomczynski and Sacchi (1987). The specific cDNA probe was labeled with [^{32}P]-deoxycytidine 5'-triphosphate ([^{32}P]-dCTP) by the random prime method. The northern blot analysis was performed as described by Jastrzebska and coauthors (2000). For western blot analysis, extracts from various rat tissues were prepared. The same amount of proteins from each extract (20 μg) was applied on the SDS gel and transferred onto the nitrocellulose filter. Western blots were performed using the anti-Sgt1 antibodies (a gift from Dr. K. Kitagawa, USA) diluted 1:1000.

Table 1

Expression of Sgt1 in the rat tissues as studied by the northern and western blots

Tissue	mRNA for Sgt1 level	Sgt1 protein level
brain	+ +	+ + +
lung	+ + +	+ +
skeletal muscle	+ + +	+ + +
heart	+ + +	+ +
stomach	+	+
spleen	+ + +	+ + +
kidney	+ +	+ +
liver	+ +	+ +

Northern blot was performed as described by Jastrzebska and coauthors (2000), using a specific cDNA probe. Western blot containing 20 μg of protein extract from each tissue was developed with specific polyclonal anti-Sgt1 antibodies. (+++) high level; (++) moderate level; (+) low level of Sgt1 protein or mRNA.

As it is seen in the Table I, Sgt1 mRNA and protein are present in all tissues examined with the highest level in the brain, skeletal muscles and spleen. The high expression of Sgt1 in rat brain tissues is in agreement with the data obtained for human Sgt1 (Niikura and Kitagawa 2003, Zou et al. 2004) and for the Sgt1 homologue, CacyBP/SIP (Jastrzebska et al. 2000). To analyze the presence of Sgt1 in various areas of the rat brain we performed western blots using 15 μg of protein extract isolated from the cortex, cerebellum and hippocampus. Results of that analysis showed that cerebellum and cortex contain high amounts of Sgt1 protein (Fig.1).

To find in which brain cells the Sgt1 protein is present we performed immunohistochemistry on paraffin sections of the rat brain. The brains were processed in a standard manner including immersion-fixation in 8% phosphate buffered formaldehyde, embedding in paraffin and cutting with a microtome into 20 μm sections. Prior to immunohistochemical labeling with the anti-Sgt1 polyclonal antibodies (diluted 1:50), sections were dewaxed with xylene and ethanol. The labeling was

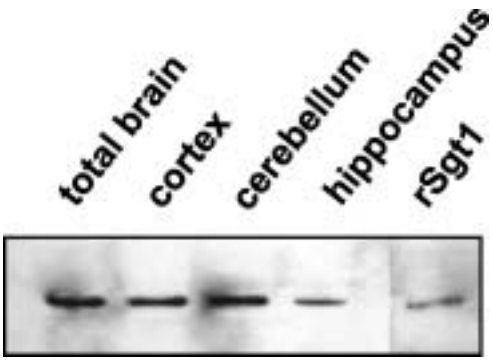

Fig. 1. Western blot developed with polyclonal antibodies showing Sgt1 levels in some areas of the rat brain. 15 µg of each protein extract was applied on the gel. rSgt1, 5 ng of human recombinant protein was used as a standard.

visualized with 3,3' - diaminobenzidine. To control specificity of the immunoreaction some sections were incubated without the primary antibody. The immunohistochemical labeling showed that high amounts of the Sgt1 protein are present in some populations of neurons like the Purkinje cells of the cerebellum and in the glial cells of white matter (Fig. 2). Although these results showed that the Sgt1 protein is abundant in the brain, it is difficult to speculate what are its roles in this tissue.

Kitagawa and coauthors (1999) suggested that the structure of Sgt1 is conserved in evolution and this is in agreement with database screening analysis showing that Sgt1 gene is conserved among the eukaryotic organisms. Both plant and mammalian Sgt1 protein is composed of several domains: N-terminal TPR domain containing tetra-tricopeptide repeat motifs, CS domain localized in the middle of the molecule (present in CHORD-containing proteins and Sgt1) and SGS domain (Sgt1 specific) in the C-terminal part of the Sgt1 molecule. Analysis of the recombinant human Sgt1 revealed that SGS domain of Sgt1 has 30% identity in the amino acid sequence with the C-terminal fragment of mouse and human CacyBP/SIP (Filipek and Kuznicki 1998, Matsuzawa and Reed 2001). In our previous work we showed that within the SGS domain the fragment responsible for binding of S100 proteins is located (Nowotny et al. 2003). The SGS domain binds not only the members of the S100 family but also Cyr1p/Cdc35p proteins (Dubacq et al. 2002).

The TPR and CS domains of Sgt1 bind Skp1 and some heat shock proteins. In collaboration with Dr. W. Chazin's group we found that human Sgt1binds HSP90 (Lee et al. 2004). Data obtained for yeast Sgt1 showed that Sgt1 interacts with member of the heat shock family, Hsc82, and that this protein stimulates binding of Sgt1 to Skp1 and then their association with Ctf13 and CBF3 complex (Bansal et al. 2004). It is not clear what is the role of Sgt1 in CBF3 complex formation but it seems that Sgt1 might function as a factor that links multiprotein complex assembly with their turnover. Regulation of CBF3 by Sgt1 and Hsp90 was also studied by Lingenbach and Kaplan (2004). These authors revealed that association of Sgt1 with Skp1 depends on binding of ATP to Hsp90. Hydrolysis of ATP after the complex has been formed allows Hsp90 to keep Sgt1 in an appropriately folded state and then to associate with Skp1, which allows for their participation in the assem-

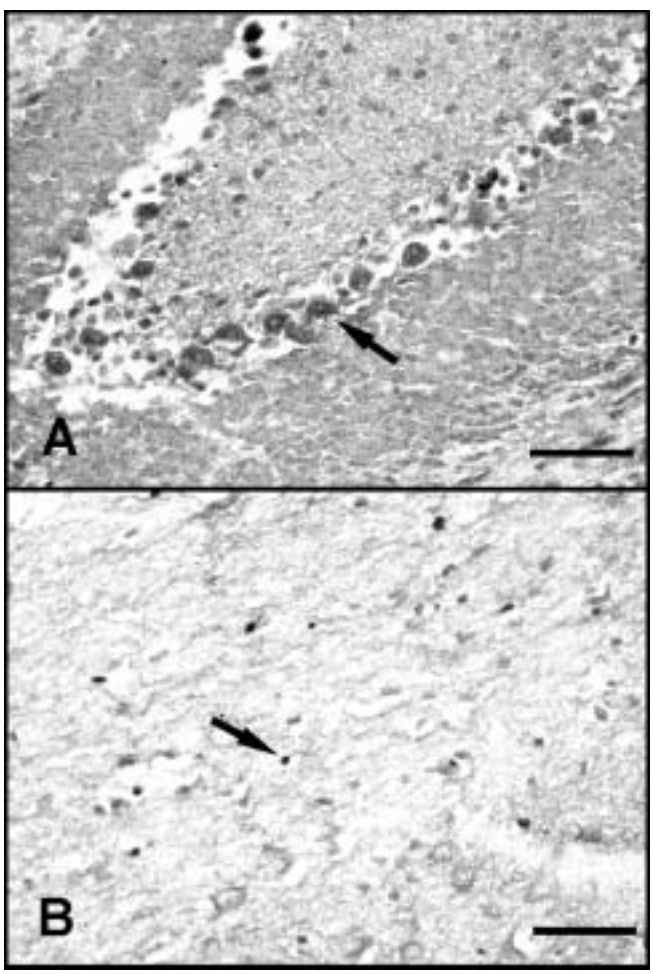

Fig. 2. Immunohistochemical localization of Sgt1 in the rat cerebellum (A) and white matter (B). In (A) immunolabeling with the anti-Sgt1 antibodies is seen in Purkinje cells and (B) in some glial cells (arrows). Bars: 50 µm.

Table II

Target proteins of Sgt1 and possible functions of the complexes formed between Sgt1 and these proteins

Target protein	Sgt1 domain	Probable function of interaction	References
Skp1	TPR +CS	- activation of multiprotein complexes CBF3 and SCF	Kitagawa et al. 1999
		- assembly of CBF3 complex and its turnover. Influence on proper kinetochore function	Rodrigo-Brenni et al. 2004
Ctf13	TPR +CS	- influence on proper CBF3 assembly	Lingelbach and Kaplan 2004
Cyr1/Cdc35	SGS	- contribution to the activity of the cAMP pathway	Dubaq et al. 2002
Rad6	?	- involvement in DNA repair system and protein degradation - cell cycle progression of meristematic cells	Yamamoto et al. 2004
RAR-1	CS	- regulation of R-gene triggered disease resistance pathway	Azavedo et al. 2002
		- activation of several components of SCF and COP9 complexes	Shirasu and Schulze-Lefert 2003
Bs2	?	- regulation of bacterial disease resistance in RAR-1 independent manner	Leister et al. 2005
HSP90	TPR + CS	- regulation of CBF3 complex assembly and turnover	Bansal et al. 2004
		- forming protein complex with Hsp90 and RAR1 to stabilise RPM1 conformation	Hubert et al. 2003
		- regulation of multiprotein complexes involved in R-gene mediated resistance	Takakashi et al. 2003 Liu et al. 2004
S100A6 S100B S100P	SGS	- unknown	Nowotny et al. 2003

bly of CBF3 complex. When the nucleotide is exchanged, Hsp90 returns to the ATP-bound form and the C-terminal structure of Sgt1 is refolded. This refolding decreases the affinity of Sgt1 to Skp1.

The proteins which were found to interact with Sgt1 and the possible importance of those interactions are shown in Table II. It seems that Sgt1 may be an element of a large complex protecting cells from various adverse conditions. However, more detailed studies concerning interactions of Sgt1 with its targets are needed to better understand the function of this protein in various cells including the cells of the central nervous system.

We thank Dr. K. Kitagawa for the sample of polyclonal antibodies against Sgt1, Dr. R. Djavadian for

her help in immunohistochemical labeling and Prof. J. Kuznicki and Dr. W. Leśniak for critical reading of the manuscript. This work was supported by the State Committee of Scientific Research grant to A.F., FIRCA to J.K. and statutory funds from the Nencki Institute of Experimental Biology.

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Received 3 March 2005, accepted 15 April 2005