FEBS Letters 586 (2012) 3805–3812

journal homepage: www.FEBSLetters.org

SAP97 promotes the stability of Na_x channels at the plasma membrane

Masahito Matsumoto [a], Akihiro Fujikawa [a], Ryoko Suzuki [a], Hidetada Shimizu [a], Kazuya Kuboyama [a], Takeshi Y. Hiyama [a,b], Randy A. Hall [c], Masaharu Noda [a,b,*]

[a] Division of Molecular Neurobiology, National Institute for Basic Biology, 5-1 Higashiyama, Myodaiji-cho, Okazaki, Aichi 444-8787, Japan
[b] School of Life Science, The Graduate University for Advanced Studies, 5-1 Higashiyama, Myodaiji-cho, Okazaki, Aichi 444-8787, Japan
[c] Department of Pharmacology, Rollins Research Center, Emory University School of Medicine, Atlanta, GA 30322, USA

ARTICLE INFO

Article history:
Received 2 August 2012
Revised 30 August 2012
Accepted 10 September 2012
Available online 25 September 2012

Edited by Maurice Montal

Keywords:
Sodium channel
PDZ array
PDZ-binding motif
MAGUK protein
Subfornical organ
Astrocyte

ABSTRACT

Na_x is a sodium-level sensor for body fluids expressed in the circumventricular organs in the brain. Na_x has a putative PSD-95/Disc-large/ZO-1 (PDZ)-binding motif at the carboxyl (C)-terminus. Here we found that several PDZ proteins bind to Na_x by PDZ-array overlay assay. Among them, synapse-associated protein 97 (SAP97/DLG1) was coexpressed with Na_x in the subfornical organ. In C6 glioblastoma cells, destruction of the PDZ-binding motif of Na_x or depletion of SAP97 resulted in a decrease in cell-surface Na_x, which was attenuated with inhibitors of endocytosis. These results indicate that SAP97 contributes to the stabilization of Na_x channels at the plasma membrane.

Structured summary of protein interactions:
Nax physically interacts with **SAP97** by anti tag coimmunoprecipitation (View interaction)
CNRasGEF binds to **Nax** by protein array (View interaction)
Nax and **SAP97** colocalize by fluorescence microscopy (View interaction)
GIPC1 binds to **Nax** by protein array (View interaction)
ZO-1 binds to **Nax** by protein array (View interaction)
SAP97 binds to **Nax** by protein array (View interaction)
Densin-180 binds to **Nax** by protein array (View interaction)
Beta-1-syntrophin binds to **Nax** by protein array (View interaction)
ERBIN binds to **Nax** by protein array (View interaction)
Nax physically interacts with **SAP97** by pull down (View interaction)
Lnx1 binds to **Nax** by protein array (View interaction)
nNOS binds to **Nax** by protein array (View interaction)

1. Introduction

Sodium (Na) is a major electrolyte of extracellular fluids and the main determinant of osmolality. Na homeostasis is essential to life and Na^+ concentrations in plasma and cerebrospinal fluid (CSF) are continuously monitored to maintain a physiological level of Na^+ in body fluids [1]. We have previously shown that Na_x, which structurally resembles voltage-gated sodium channels ($Na_v1.1$–1.9), is a concentration-sensitive Na channel with a threshold of ~150 mM for extracellular Na^+ concentration $[Na^+]_o$ in vitro [2–4].

In the brain, Na_x channels are specifically expressed in astrocytes and ependymal cells in the sensory circumventricular organs (sCVOs), such as the subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT), where Na_x-positive glial cells are involved in sensing an increase in $[Na^+]$ in body fluids [5]. *Na_x*-deficient mice do not stop ingesting salt even when dehydrated, while wild-type mice avoid salt [6]. This behavioral defect of *Na_x*-deficient mice is recovered by a site-directed transfer of the *Na_x* gene with an adenoviral vector into the SFO [7]. Na_x thus functions as the brain's Na^+-level sensor for the homeostatic control of $[Na^+]$ in body fluids.

Some PDZ domain-containing proteins are known to serve as key scaffolds for channel proteins to control their trafficking [8]. PDZ domains are 90 amino-acid protein–protein interaction modules that bind to specific C-terminal motifs in their target proteins [9]. Most of the target proteins have a conserved PDZ-binding

* Corresponding author at: Division of Molecular Neurobiology, National Institute for Basic Biology, 5-1 Higashiyama, Myodaiji-cho, Okazaki, Aichi 444-8787, Japan. Fax: +81 564 59 5845.
E-mail address: madon@nibb.ac.jp (M. Noda).

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2012.09.018

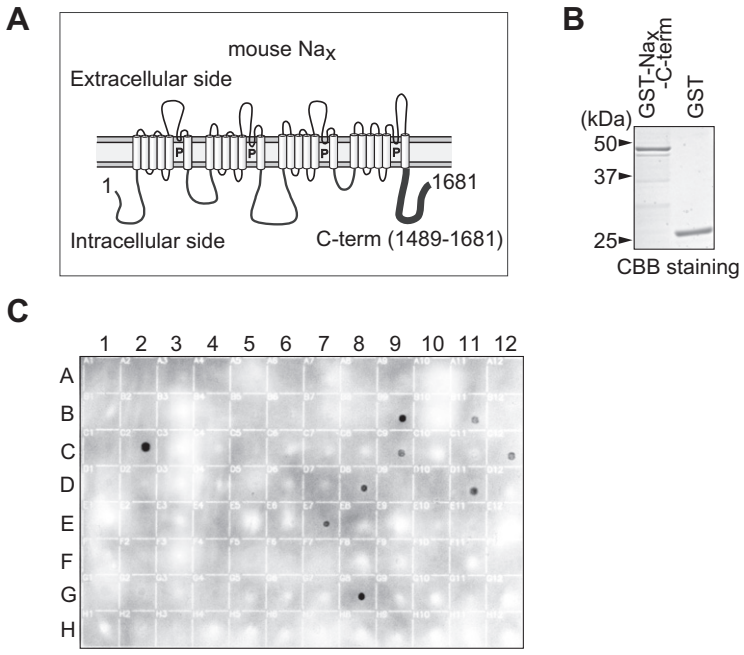

Fig. 1. Screening of PDZ proteins that bind to the C-terminus of Na_x. (A) Schematic drawing of Na_x. The C-terminal region of Na_x used for preparation of the recombinant GST-fused protein (GST-Na_x-C-term) is indicated with a bold line. (B) Preparation of GST-Na_x-C-term and control GST proteins. The purity was checked by Coomassie Brilliant Blue R-250 (CBB) staining following SDS–PAGE. (C) PDZ array overlay assay with GST-Na_x-C-term. For the proteins spotted on the array, see Supplementary Information (Table S1). Strong positive hits were observed for CNRasGEF-PDZ (B9), SAP97-PDZ1+2 (C2), and LNX1-PDZ3 (G8).

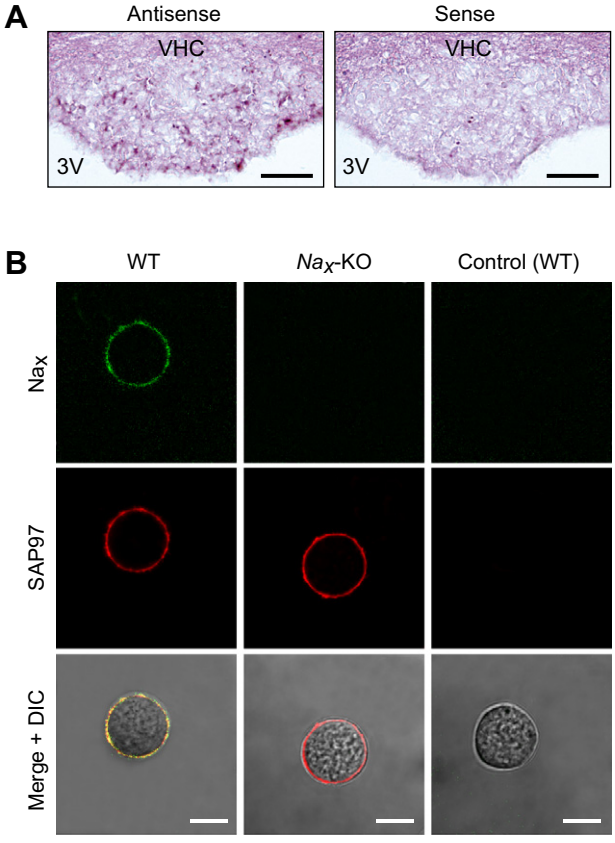

Fig. 2. Expression of Na_x and SAP97. (A) Expression of *SAP97* in the SFO. Coronal tissue sections of the mouse SFO were hybridized with antisense SAP97, or with the control sense probe. VHC, ventral hippocampal commissure; 3V, dorsal third ventricle. Scale bars, 50 μm. (B) Double immunostaining of SFO cells obtained from wild type (WT; left) and *Na_x*-knockout (*Na_x*-KO; middle) mice with anti-Na_x and anti-SAP97 antibodies. Right panels show the results of control experiments omitting the primary antibodies. DIC, differential interference contrast. Scale bars, 10 μm.

motif that matches one of the three 'canonical' consensus motifs (Class I, –X–S/T–X–L/V; Class II, –X–Ψ–X–Ψ; Class III, –X–D/E–X–L/V; Ψ indicates hydrophobic amino acid; [9]). Giallourakis et al. [9] recently found a new consensus motif (–X–S/T–X–I/A) other than the 'canonical' motifs. Because the C-terminal sequence of Na_x (–Q–T–Q–I for rat and mouse, and –Q–S–Q–I for human) fits this 'non-canonical' PDZ-binding motif, we hypothesized that the Na_x channel may be regulated by PDZ-scaffold proteins. In the present study, we took advantage of a proteomic PDZ-domain array [10], containing 96 distinct PDZ domains, to screen for PDZ proteins that might interact with Na_x. Among the PDZ proteins thus identified, we found that SAP97 (also known as DLG1) is coexpressed with Na_x in the SFO, and contributes to the stability of Na_x channels in the plasma membrane.

2. Materials and methods

2.1. Recombinant proteins

The Glutathione S-transferase (GST)-fused protein with the C-terminal region (amino acid residues 1489–1681) of mouse Na_x (GST-Na_x-C-term), its PDZ-binding-motif mutant (GST-Na_x-C-term-T1679A) in which Thr-1679 was replaced with Ala, or its PDZ-binding-motif deletion mutant (GST-Na_x-C-termΔTQI) was expressed in an *Escherichia coli* strain, BL21, and purified by glutathione affinity chromatography as described [11].

2.2. Overlay assay on the PDZ array

The PDZ-array overlay assay was performed as described [10,12]. Briefly, a nylon membrane spotted with a series of His/S-tagged PDZ domain proteins was pre-treated with a blocking buffer containing 2% non-fat dry milk and 0.1% Tween-20 in 150 mM NaCl and 10 mM phosphate buffer, pH 7.3 (PBS) for 30 min, then overlaid with the GST-fused proteins (15 nM) in the blocking buffer.