P-I-1

Subcellular localization of HAP1-GFP fusion protein in the cultured astrocytes

Mamoru Nagano[1], Atuko Fuzioka[1] and Koh Shinoda[2]

[1]Department of Anatomy, Kinki University school of Medicine

[2]Department of Anatomy, Yamaguchi University school of Medicine

HAP1 was identified as a huntingtin-associated protein and has expressed highly in brain. There are at least two subtypes of HAP1, HAP1A and HAP1B, which have different C-terminal amino acid suquence. The function of HAP1 is unknown. In this study, we examined the subcellular localization of HAP1 wigh HAP1-GFP fusion protein. Four kinds of expression plasmid of HAP1-GFP were constructed: the plasmids to express HAP1A and HAP1B at the site of the C-terminus of GFP, and those to express at the site of its N-terminus. These plasmid were introduced into astrocytes and were observed under a fluorescence microscope or a laser scanning confocal microscope. The expression of fusion proteins was assessed by a Western blotting. Transfection of astrocytes with HAP1A-GFP resulted in numerous dot-like structures in the cytoplasm. Whereas, the fluorescence of HAP1B-GFP were diffusely distributed in the cytoplasm of transfected astrocytes. This evidence suggest that HAP1A is essential to the formation of this structure. Electron microscopic analysis showed that these dot-like structures appears as a non-membrane bounded cytoplasmic inclusion, approximately 1-3 um in diameter, which has a granulo-fuzzy texture with moderate-to low electron density. This structure is very similar to stigmoid body that we previously indentified. HAP1-GFP fusion protein showed similar localization to its of α-tubulin. These results support that HAP1 may have a role in intracellular trafficking. Occasionally large green fluorescent aggregates grew in the cytoplasm of the HAP1-GFP transfected cells. The significance is not clear.

P-I-2

Subcellular distribution of estrogen receptor α visualized with green fluorescent protein

Maki YOSHIDA[1,2], Mayumi NISHI[1], Zenro KIZAKI[2], Tadashi SAWADA[2], Mitsuhiro KAWATA[1]

[1]Department of Anatomy and Neurobiology and [2]Pediatrics, Kyoto Prefectural University of Medicine, Kyoto

Estrogen receptor α (ERα) is a member of a nuclear receptor superfamily and exists not only in the peripheral reproductive tissues but also the central nervous system influencing neuroendocrine function and neuronal differentiation. ERα binds to the genomic DNAs and determines specific gene expressions as a transcription factor. It is generally recognized that ERα resides in the nucleus, but some investigations with the procedures of immunocytochemistry (ICC) or cell enucleation method showed its distribution in both nucleus and cytoplasm.

In this study, GFP was fused at the amino-terminal of a rat ERα that lacked the first 58 amino acid residues and GFP-ERα chimera protein was constructed. We performed the ICC, immunoblotting analysis, and promoter assay with the transiently transfected COS-1 cells. COS-1 cells expressing GFP-ERα were analyzed using epifluorescence microscope which showed its nuclear localization and no translocation between the nucleus and cytoplasm under the influence of estradiol (E_2) or Tamoxifen (Tam). Intranuclear distribution and the relation between ERα and chromatin in transfected cells were investigated with confocal laser scanning microscope using propidium iodide (PI). Transfected cells showed diffusely dispersed fluorescence of GFP-ERα in the nucleus without ligands. Cells presented nuclear redistribution of GFP-ERα to punctate pattern with E_2-treatment, while only a part of cells were recognized their redistribution with the treatment of Tam. There was no positive correlation of the distribution pattern between GFP-ERα and PI with ligand treatment, indicating that the cluster of GFP-ERα does not simply reflect the site of transcription as seen for euchromatin.

P-I-3

Localization of GFP-Fused Calbindin D 28k in MDBK cells.

Atsuko Fujioka and Mamoru Nagano

Department of Anatomy, Kinki University School of Medicine, Osaka-Sayama

Calbindin D 28k in the Ca-absorbing cells has been supposed to act as a diffusional facilitator for Ca-transport, as a buffer for maintaining intracellular Ca levels, and as an activator of Ca^{2+}-ATPase. The exact functions, however, have yet to be resolved. The present study was attempted to elucidate the translocation of calbindin D 28k tagged with EGFP in the overexpressed MDBK cells. Two kinds of expression plasmid of pCal D-GFP were constructed: one was the plasmid to express calbindin D 28k at the site of the C-terminus of GFP (Cal D-C), and another was one to express at the site of its N-terminus (Cal D-N). These plasmids were introduced into MDBK cells by an electro-poration method. The expression of fusion proteins was assessed by a Western blotting. The fusion protein-overexpressed cells were observed under a fluorescence microscope or a laser scanning confocal microscope, at 0-20 min. after ionomycin or parathyroid hormone (PTH) administration. An immunocytochemical procedure using anti calbindin D 28k antibody for a post-embedding method was applied to the overexpressed cells for the intracellular localization. Both the Cal D-N expressing and the Cal D-C expressing cells showed intense fluorescence throughout the cytoplasm. There were, however, differences in expression levels in nuclei. Intense fluorescence in the nuclei was seen in the Cal D-N expressing cells, but a little in the Cal D-C expressing cells. The present study focused on the redistribution of Cal D-C in the cytoplasm associated with the increase of $[Ca^{2+}]i$. The expression of cal D-C was identified by the Western blotting. The fluorescence of the Cal D-C showed a network pattern in the cytoplasm around the nucleus in 5-90 sec. of the addition of parathyroid hormone or ionomycin, which induced the increase of $[Ca^{2+}]i$. The network pattern seemed to correspond to the distribution of endoplasmic reticulum . Aggregations of immuno-gold showing overexpressed calbindin D 28k, observing the localization of calbindin D 28k under an electron microscope, appeared on the ER, mitochondria (M) microfilament and nucleus. The role in the activator for Ca^{2+} ATPase is considered like its role in cell membrane, because both the ER and mitochondria sequester Ca^{2+} by Ca^{2+}-ATPase.

P-I-4

Nonviral gene transfer into the neurons by microelectroporation

Kiyoshi KUROKAWA, Maki MURATA, Hisao YAMADA, Motoi KUDO

Department of Anatomy, Shiga University of Medical Science, Otsu

It is important to develop techniques for efficient gene transfer into intact neural tissues. In this study, we developed new nonviral method for gene transfer to the rat neurons. The DNA encoded the cholera toxin B subunit (CTb) was inserted into the *NheI*/*Sac*II site of the plasmid pQBI25, which is green fluorescence protein (GPF) expression vector. The male Wister rats (bw: 160-200g) were anesthetized with sodium pentobarbital (50mg/kg. i.p.) and placed in a stereotaxic apparatus. Three holes were drilled in the skull. The plasmid DNA was injected via the center hole using the Hamilton syringe. The anode and cathode microelectrodes, which were made from the tungsten wire, were inserted from the bilateral holes. The anode and cathode were connected to the square-wave electroporator BTX T820. A series of two electro pulses (each 50ms induration) was admininistered at voltage of 10V. After the 4 days, the animals were deeply anesthetized with pentobarbital (50mg/kg, i.p.), and perfused transcardially with PBS and subsequently with 4% formaldehyde and 0.2% picric acid in PB. After the postfixation, the brain was cut at 30 μm thick coronal sections in a cryostat. The sections were incubated with goat anti-CTb antibody. The sections were reacted with biotinylated rabbit anti-goat IgG antibody followed by Texas red conjugated avidin. In the hippocampus, the neurons expressed GFP were observed, and almost all were immunostained by using the anti-CTb antibody. The present study demonstrates microelectroporation (the low voltage and wide pulse method) is the effective for gene transfer.