Expression of Sugar Transporters by In Vivo Electroporation and Particle Gun Methods in the Rat Liver: Localization to Specific Membrane Domains

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To see the cellular localization mechanism of membrane proteins in the hepatocyte in situ, we introduced cDNAs of facilitated-diffusion sugar transporters, GLUT1, GLUT3, GLUT4, and GLUT5, and a Na⁺-dependent active sugar transporter SGLT1 into the rat liver by the in vivo electroporation method and the particle gun method, and the localization of their products was analyzed by immunofluorescence microscopy. SGLT1 was strictly restricted to the bile canalicular (apical) domain, whereas GLUT1 was found in the sinusoidal (basolateral) membrane of hepatocytes. GLUT3 and GLUT5 were present along the whole aspects of the plasma membrane with a tendency for the bile canalicular membrane to be enriched with transporters as compared with the sinusoidal membrane. GLUT4 remained in the intracellular compartments. Simultaneous expression of two of the transporters confirmed these results. Compared with membrane localization of sugar transporters in MDCK cells, GLUT1, GLUT4 and SGLT1 exhibited a similar localization pattern. On the other hand, localization of GLUT3 and GLUT5 was different from that in MDCK cells. These observations suggest that hepatocytes in situ may have a different localization mechanism for sugar transporters from that in MDCK cells. In addition, the in vivo electroporation and the particle gun methods seem to be useful tools for the introduction and analysis of foreign genes in the liver in situ.

Key words: GLUT, SGLT1, Canalicular membrane, Sinusoidal membrane, Immunohistochemistry

I. Introduction

Hepatocytes are specialized epithelial cells of endodermal origin, which carry out diverse functions such as synthesis and secretion of serum proteins and formation of bile. The hepatocytes are polarized and their plasma membranes are divided into two distinct domains: bile canalicular (apical) and sinusoidal (basolateral) domains [54, 57]. Tight junctions surrounding bile canaliculi separate the canalicular and sinusoidal domains. One of the characteristic features of hepatocyte polarization is that a single hepatocyte usually has two to three separate canalicular domains, since a bile canaliculus is formed between two hepatocytes and each hepatocyte usually lies in contact with two to three hepatocytes in the hepatic cell cord [54]. Apart from these morphological differences from the ordinary epithelial cells such as kidney tubule cells and enterocytes, hepatocytes are unique in their trafficking mechanism of newly-synthesized membrane proteins. Most canalicular membrane proteins are first delivered to the sinusoidal domain and then targeted to the canalicular membrane by transcytosis, which makes a marked contact to a direct delivery mechanism typically observed in MDCK cells [19, 23, 30, 54, 57]. Sugar transporters are membrane proteins that mediate the transport of sugars across the plasma membrane. Two types of sugar transporters have been found in mammalian cells, facilitated-diffusion sugar transporters (GLUT family) and Na⁺-dependent active sugar transporters (SGLT family) [2, 5, 11, 43]. GLUT sugar transporters facilitate the transport of sugars across the membrane according to the concentration gradient of their
substrates. Five isoforms have been identified so far: GLUT1, GLUT2, GLUT3, GLUT4, and GLUT5 [2, 5, 43]. SGLT sugar transporters are Na⁺-coupled concentrative cotransporters driven by the electrochemical gradient of Na⁺ across the plasma membrane, which is generated by Na⁺-K⁺ ATPase by the expense of ATP. At least three isoforms, SGLT1, SGLT2, and SGLT3 have been reported [11, 12]. These sugar transporters exhibit cell-type-specific expression in a variety of tissues and organs. In muscle cells and adipocytes whose glucose uptake is regulated by insulin, GLUT4 is sequestered into the cytoplasmic vesicles and tubules [6]. Upon insulin stimulation, this cytoplasmic GLUT4 is translocated to the plasma membrane, where it plays a pivotal role in lowering the blood glucose level. In epithelial cells, sugar transporters are localized to distinct membrane domains, apical and basolateral plasma membranes, which are crucial in the vectorial transfer of sugars, thereby maintaining specific functions of each organ, such as sugar absorption in the small intestine [40, 42, 47, 49, 53].

In the rat liver, GLUT2 is endogenously expressed and localized at the sinusoidal plasma membrane of hepatocytes [51] and plays an important role in blood glucose homeostasis [48]. Such polarized localization of membrane proteins has been studied in the cultured epithelial model cell systems such as MDCK cells [8, 19, 23]. In hepatocytes, establishment of the polarized hepatocyte system in culture was difficult and hepatocyte couplets were sometimes used [54]. Expression of exogenous genes in the liver of living animals, therefore will provide a clue as to whether the results obtained in these culture model systems directly reflect the cellular and molecular events occurring in the hepatocytes in the liver in situ.

Electroporation has been used in living animals to deliver anti-cancer drugs for therapeutic purposes [3, 4, 13, 15, 18, 25] as well as to introduce nucleic acids and proteins into organs [17, 20, 26, 29]. The electroporation technique has been successfully used to introduce foreign genes into the liver in situ [14]. Using disk-shaped electrodes, we examined the parameters that might affect the efficiency of electro-transfer of foreign DNA by digital analysis of microscopic images using green fluorescent protein (GFP) [39].

Delivery of foreign genes by the particle gun method is unique in that genes are introduced into the cells with small gold particles of high velocity [20]. Development of a hand-held-type machine [36, 56] allowed us to use this method for the in vivo expression of foreign genes in the rat liver.

In order to shed light on the localization mechanism of membrane proteins in hepatocytes, we directly introduced plasmid DNAs of five different sugar transporters, i.e., GLUT1, GLUT3, GLUT4, GLUT5, and SGLT1, into the rat liver in situ by the in vivo electroporation and particle gun methods. Expression and localization of transfected gene products were analyzed immuno-

II. Materials and Methods

Animals

Male Wistar rats of 6 weeks of age weighing approximately 100–120 g supplied from the Animal Breeding Facility, Gunma University (Gunma, Japan) were used. All the procedures were in accord with the "Guide for the Care and Use of Laboratory Animals" of the National Academy of Sciences.

Plasmid DNAs of sugar transporters

The cDNA clone of rat GLUT4 was a generous gift from Dr. Kasahara (Tokyo University) (Kasahara and Mori, GenBank D16101). The cDNA clones of rat SGLT1 [37], rabbit GLUT1 [16], rat GLUT3 [24], and rat GLUT5 [16] were described previously. The EcoRI DNA fragments (for SGLT1, GLUT3, GLUT4, GLUT5) and the HindIII DNA fragment (for GLUT1) all including the entire open reading frame of cDNA clones, were ligated into the pcDNA3 expression vector (Invitrogen, Groningen, The Netherlands). pEGFP-C1 was from Clontech (Palo Alto, CA). Plasmid DNAs were multiplied by culturing the host bacteria JM109 and isolated with a Qiagen plasmid DNA isolation kit (Qiagen, Santa Clarita, CA). They were dissolved in phosphate-buffered saline (PBS) at a concentration of 1 μg/pl.

Electric pulse generator and electrodes

An Electro Square Porator (T820; BTX, San Diego) with a pair of tweezer-type electrodes whose tips consisted of 1 cm diameter disks (model 449–10 PRG; Meiwa Shoji, Tokyo, Japan) [39] (Fig. 1) was used. The electric resistance of the liver was monitored with a graphic pulse analyzer (MVC540R, Meiwa Shoji).

Fig. 1. Introduction of sugar transporters into the rat liver by in vivo electroporation. The left lateral lobe of the liver was sandwiched with a pair of electrode disks, and electric pulses were applied immediately after the plasmid injection. Bar = 1 cm.
Procedure of in vivo electroporation

Rats were divided into eleven groups, each consisting of three animals. Five groups were used for the transfection of plasmid DNAs harboring sugar transporters, i.e., GLUT1, GLUT3, GLUT4, GLUT5, and SGLT1. The other five groups received cotransfection of GLUT1/GLUT4, GLUT3/GLUT4, GLUT5/GLUT4, GLUT1/GLUT3, and GLUT1/GLUT5 plasmids. One group received pEGFP-C1 only and served as a control. Electric gene transfer was carried out basically as described previously [39]. In short, the rats were anesthetized with diethyl ether, a small left hypochondrial surgical incision about 1.5 cm was made, and the left lateral lobe was exposed out of the abdominal cavity. The edge of the liver lobe was caught with a pair of tweezer-type electrode disks. Each 100 μg of five kinds of plasmid DNAs (GLUT1, GLUT3, GLUT4, GLUT5, and SGLT1) in 100 μl of PBS was directly injected into the liver between the two electrodes with a 27-gauge needle. To obtain a high level of expression, 100 μg of DNA was used for a single transfection of each sugar transporter gene since transfected gene expression increased as the amount of injected DNA increased [39]. In the cotransfection experiments, 50 μg each of DNA was used. Immediately after the DNA injection, electric pulses were applied under the conditions as optimized previously: 50 V at voltage intensity, 50 ms in duration, 4 pulses with 1-second interval between pulses [39]. In order to minimize the tissue damage, the number of pulses was reduced from 8 to 4 as compared with our previous study [39]. Electroporated liver was returned to the abdominal cavity and the abdominal wound was sutured layer to layer to the abdominal muscle and the skin.

Particle gun delivery method

A hand held type particle gun system, Helios Gene Gun system (Bio-Rad Laboratories, Hercules, CA) was used. Coating of gold particles (diameter: 1.6 μm) with pEGFP-C1 or a plasmid of either of sugar transporters and preparation of gold-coated cartridges was carried out according to the manufacturer’s protocol using Tefzel tube (Bio-Rad) and Tubing Prep Station (Bio-Rad). The coated cartridges were stored at −20°C until use. Three rats were used for the transfection of each sugar transporter. The animals were anesthetized with diethyl ether, and the left median lobe of the liver received three consecutive shots at a gas pressure of 250 psi.

Tissue preparation

Two days after in vivo transfection, rats were anesthetized with diethyl ether and killed by cervical dislocation. The lobes treated with electroporation or particle gun shots were resected and sampled. The specimens were cut into slices at a thickness of about 2 mm with an array of razor blades in cross direction against the electrode disks. They were fixed in 2% paraformaldehyde (PFA) in 0.1 M sodium phosphate buffer, pH 7.4, for 2 hr on ice and washed with PBS. They were infused with 20% sucrose in 0.1 M sodium phosphate buffer, pH 7.4, containing 0.02% sodium azide at 4°C overnight, embedded in Tissue Tek OCT compound (Sakura Finetechical, Tokyo, Japan), rapidly frozen with liquid nitrogen, and stored at −80°C until use.

Immunohistochemistry

Cryostat sections of 10 μm thickness were cut from specimens fixed in 2% PFA, mounted on poly-L-lysine-coated glass slides, and washed with PBS. Immunofluorescence staining was carried out basically as previously described [31, 32]. In short, sections were treated with 5% normal goat serum in PBS at room temperature for 20 min. Sections were incubated with each of the anti-sugar transporter antibodies at room temperature for 1 hr. Anti-sugar transporter antibodies used were, rabbit anti-GLUT1 (1: 500 dilution) [44], rabbit anti-GLUT2 (East Acres Biologicals, Southbridge, MA, USA, 1: 500 dilution), rabbit anti-GLUT3 (1: 1000 dilution) [31], rabbit anti-GLUT4 (1: 500 dilution) [41], rabbit anti-GLUT5 (1: 500 dilution) [16], and rabbit anti-SGLT1 (1: 200 dilution) [45]. After being washed with PBS, the sections were incubated with DTAF (dichlorotriazinylamino fluorescein)-labeled donkey anti-rabbit IgG (Jackson Immunoresearch, West Grove, PA, USA, 1: 200 dilution) as a secondary antibody at room temperature for 1 hr. Rhodamine-phalloidin (Molecular Probes, Eugene, OR, USA) and 2 μg/ml TO-PRO-3 iodide (TO-PRO-3, Molecular Probes, Eugene, OR, USA) were included in the secondary antibody solution for F-actin and DNA counterstaining, respectively [22, 38]. After being washed with PBS, the sections were mounted in a mounting medium comprised of 22% polyvinylalcohol (Wako, Japan), 11% glycerol, 56 mM Tris-HCl buffer, pH 9.0 containing 5% DABCO (1,4-diazabicyclo [2, 2, 2] octane) as an anti-bleaching reagent [32]. The sections were examined with a BX-50 microscope (Olympus, Tokyo, Japan) equipped with an MRC-1024 laser confocal system (Bio-Rad) utilizing a 15-mW krypton/argon laser. In some cases, 2 μg/ml DAPI (4,6-diamidino-2-phenylindole dihydrochloride) (Boehringer-Mannheim, Mannheim, Germany) was used instead of TO-PRO-3 and sections were examined with an AX-70 epifluorescence microscope (Olympus). Images were recorded with a PXL1400 cooled-CCD camera (Photometrix, Tucson, AZ, USA).

In the cotransfection experiments, double-immunofluorescence staining was performed using a mixture of appropriate anti-sugar transporter antibodies raised in rabbit and guinea pig as primary antibodies. A mixture of DTAF-labeled donkey anti-guinea pig IgG (Jackson Immunoresearch, 1: 200 dilution) and LRSC (lissamine rhodamine sulfonyl chloride)-labeled donkey anti-rabbit IgG (Jackson Immunoresearch, 1: 200 dilution) containing TO-PRO-3 was used as secondary antibodies.
III. Results

Localization of endogenous GLUT2

Presence of GLUT2 which is endogenously expressed in hepatocytes was examined by laser confocal microscopy. GLUT2 was localized at the sinusoidal membrane domain in hepatocytes (Fig. 2a). Bile canaliculi corresponding to the apical membrane domain, which were clearly demarcated with F-actin by rhodamine-phalloidin staining, were negative for GLUT2. Cells other than hepatocytes were negative for GLUT2. These observations are in accord with those of Thorens et al. [51], confirming the polarized sinusoidal distribution of GLUT2 in rat hepatocytes.

Localization of in-vivo-transfected GLUT1, GLUT3, GLUT4, GLUT5, and SGLT1

Rat livers were transfected with plasmids harboring sugar transporters, GLUT1, GLUT3, GLUT4, GLUT5, and SGLT1 by in vivo electroporation, and their expression and localization were examined. Two days after the in vivo electroporation, expression of these exogenously introduced sugar transporters was evident. Their localization was highly reproducible in hepatocytes.

Positive signals for GLUT1 were confined to the sinusoidal membrane in hepatocytes (Fig. 2b). Bile canalicular membrane was not labeled for GLUT1. Positive signals for GLUT3 and GLUT5 were distributed along the whole aspects of the plasma membrane, i.e., at the canalicular and sinusoidal membranes in hepatocytes (Fig. 2c, d). Positive signals for GLUT4 were localized not at the plasma membrane but within the cytoplasm (Fig. 2e, f). In the SGLT1-transfected livers, positive signals for SGLT1 were localized along the canalicular membrane in hepatocytes (Fig. 2h), although successful introduction of SGLT1 into hepatocytes was observed only in a few hepatocytes. Instead, positive signals were commonly found at the apical membrane in duct epithelial cells in interlobular connective tissues (Fig. 2g).

By the use of the particle gun delivery method, the gold particles reached only the cells located close to the liver surface, and hence the successful expression of sugar transporters was limited to cells located in these regions. Apart from these points, the localization of sugar transporters in hepatocytes was the same as that seen by the in vivo electroporation method (Fig. 2f).

Cotransfection of GLUT1/GLUT4, GLUT1/GLUT3, GLUT5/GLUT4, GLUT3/GLUT4, and GLUT1/GLUT5 by in vivo electroporation

To further confirm the specific localization of sugar transporters in hepatocytes, cotransfection experiments with GLUT1/GLUT4, GLUT3/GLUT4, GLUT5/GLUT4, GLUT1/GLUT3, and GLUT1/GLUT5 were performed by in vivo electroporation. Introduced sugar transporters were clearly observed by double-immunofluorescence staining although the efficiency of successful co-expression of both transporters was lower compared with that in the transfection of a single sugar transporter. In GLUT1/GLUT4 cotransfection, GLUT1 was localized at the cell membrane whereas GLUT4 remained in the cytoplasm (Fig. 3a). In GLUT3/GLUT4 cotransfection, GLUT3 was localized at the cell membrane and GLUT4 in the cytoplasm (Fig. 3b). In GLUT5/GLUT4 cotransfection, GLUT5 was at the cell membrane and GLUT4 in the cytoplasm (Fig. 3c). These cotransfection studies confirmed that GLUT1, GLUT3, and GLUT5 are delivered to the plasma membrane, whereas GLUT4 remains in the cytoplasmic compartment. In GLUT1/GLUT3 and GLUT1/GLUT5 cotransfection experiments, GLUT1 was localized at the sinusoidal membrane. GLUT3 and GLUT5 were present along the whole aspects of the plasma membrane but they were much more concentrated at the canalicular domain as compared with the sinusoidal domain (Fig. 3d, e). These observations showed that GLUT3 and GLUT5 were present along the whole surface of plasma membrane, and they tended to be concentrated at the canalicular membrane. GLUT1, on the other hand, was restricted to the sinusoidal domain. The localization patterns of sugar transporters in hepatocytes are summarized in Fig. 3f.

IV. Discussion

Localization of transfected sugar transporters in hepatocytes

Hepatocytes are specialized epithelial cells and have two distinct membrane domains, i.e., bile canalicular, and sinusoidal domains, which are separated by tight junctions [30, 54, 57]. Sugar transporters exhibit distinct membrane

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Fig. 2. Immunofluorescence localization of endogenous (a) and exogenous (b-h) sugar transporters in the rat liver. Sugar transporters were introduced either by in vivo electroporation (b-e, g, h) or by the particle gun method (f). In (a)-(g), sugar transporters were stained with specific antibodies and are expressed in green. F-actin was simultaneously stained with rhodamine-phalloidin and is shown in red. Nuclei were stained with TO-PRO-3 and are shown in blue. Confocal images are illustrated. In (h), a section was double-labeled for SGLT1 (green) and GLUT2 (red), and counterstained with DAPI for nuclei (blue) and recorded with a cooled-CCD camera. Bars = 10 μm. (a) Endogenous GLUT2. GLUT2 (green) is localized along the sinusoidal domain of hepatocytes (arrowheads). Cells other than hepatocytes are negative for GLUT2. Canalicular domain densely labeled for F-actin (arrows) is negative for GLUT2. (b) GLUT1 is localized at the sinusoidal domain (arrowheads) (green). The canalicular domain is negative for GLUT1 (arrows). (c) GLUT3 (green) is present along the entire aspects of the plasma membrane of a hepatocyte, sinusoidal (arrowheads) and canalicular (arrows) domains. (d) GLUT5 (green) is present along the sinusoidal (arrowheads) and canalicular (arrows) domains. (e, f) GLUT4 (green) is retained in the cytoplasm (double-arrowheads) and the plasma membrane is negative. Introduction of GLUT4 by in vivo electroporation (e) and by particle gun method (f) gives the same localization pattern. (g) SGLT1 (green) is localized at the apical membrane of duct cells (arrows). (h) Localization of SGLT1 is restricted to the canalicular domains (arrow) of hepatocytes. Note that the sinusoidal domain is strongly labeled with GLUT2 (red).
localization in polarized cells in a variety of organs in the body [40, 47, 49]. In the present study, we introduced five sugar transporters, i.e., GLUT1, GLUT3, GLUT4, GLUT5, and SGLT1, into living rat liver in situ by in vivo electroporation and particle gun methods and compared their localization in cultured epithelial cells. Among five types of sugar transporters introduced into the liver in situ, Na+–dependent concentrative sugar transporter SGLT1 was localized exclusively to the canicular domain in hepatocytes. In epithelial cells lining the bile duct, SGLT1 was also localized at the apical membrane. Apical localization of endogenous SGLT1 has been observed in the kidney [45] and the small intestine [46] in vivo. In MDCK cells, we showed SGLT1 was localized at the apical plasma membrane after the completion of the tight junction network of occludin [37]. These results indicate that apical localization of SGLT1 is strictly controlled irrespective of the epithelial cell-types expressed.

GLUT1 expressed in the hepatocytes was present in the sinusoidal domain. In the stably-transfected Caco-2 and MDCK cells, GLUT1 was localized at the basolateral membrane [16, 27]. These observations show that GLUT1 localizes in the same membrane domain in hepatocytes in situ as in these cultured epithelial cells. In epithelial cells in the body, GLUT1 is localized at the basolateral membrane in kidney tubule cells [45, 50] and at the apical and basolateral membranes in cells of blood-tissue barriers [42]. Since transfected GLUT1 was localized at the sinusoidal membrane in hepatocytes, the localization mechanism of GLUT1 may be similar in hepatocytes and kidney cells.

GLUT5 expressed in hepatocytes in situ distributed along the whole aspects of the plasma membrane, with a tendency that canicular domain was strongly labeled compared with the sinusoidal domain. In epithelial cells of the small intestine and the kidney, GLUT5 is localized at the apical membrane like SGLT1 [21, 35, 40]. Endogenous and exogenously-introduced GLUT5 were localized at the apical brush borders in Caco-2 [16, 21]. When expressed in MDCK cells, GLUT5 was also localized to the apical membrane [27, 47]. Such apical localization makes a marked contrast to the localization of GLUT5 expressed in hepatocytes in situ in the present study. Similar results were obtained for GLUT3. GLUT3 expressed in MDCK cells [27] and endogenous GLUT3 in Caco-2 cells [10] were localized to the apical membrane, whereas expressed GLUT3 in hepatocytes in situ observed in the present study resided along the whole plasma membrane although the canicular domain was strongly labeled compared to the sinusoidal domain.

GLUT4 exhibits a unique distribution in the muscle cells and adipocytes; it is sequestered mainly to the intracellular storage compartment and translocated to the plasma membrane by insulin stimulation [6]. When GLUT4 was expressed in other cell-types in culture such as CHO cells [1], MDCK cells [27], and PC12 cell [52], it was localized not at the cell surface but in the intracellular compartment. Although the characterization of these GLUT4-bearing compartments remains to be done, GLUT4 is retained in the cytoplasmic vesicles irrespective of the cell-types expressed, suggesting that hepatocytes and other cells may utilize a common mechanism to sequester GLUT4 to the cytoplasmic compartment [52].

Endogenous liver sugar transporter GLUT2, which is a low-affinity facilitated-diffusion sugar transporter, is localized at the sinusoidal domain and is considered to be responsible for the transfer of glucose between the blood and the hepatocytes [49, 51]. The polarized localization of GLUT2 and exogenously introduced sugar transporters suggests that hepatocytes are capable of localizing sugar transporter proteins to distinct membrane domains. The mechanism for polarized localization of membrane proteins including sorting, trafficking, and retention may differ from one protein to another and from one type of cells to another, and may be regulated in different ways [7, 8, 33, 54, 55, 57]. In the polarized cell and even in the non-polarized cell, the main sorting site for newly synthesized proteins in the biosynthetic pathway is the trans-Golgi network (TGN), where routes to the apical membrane, the basolateral membrane, and the endosomal system emerge [19, 34]. In addition, secretory granules for regulated secretion are formed in the TGN. In MDCK cells most of the apical and basolateral proteins are delivered directly from the TGN to their correct membrane domains, where they may be retained by the specialized retention mechanism. Another sorting site in the biosynthetic pathway is the endosomes [19, 23, 54]. In hepatocytes, canicular membrane proteins are delivered first to the sinusoidal membrane and then transcytosed via the endosomal compartment to the canicular membrane.

Fig. 3. Localization of sugar transporters by cotransfection. Double-immunofluorescence localization of expressed transporters in the GLUT1/GLUT4 (a), GLUT3/GLUT4 (b), GLUT4/GLUT5 (c), GLUT1/GLUT3 (d), and GLUT1/GLUT5 (e) co-transfected livers. Nuclei were counterstained with TO-PRO-3 and are shown in blue. Bars = 10 μm. (a) GLUT1 (red) is present along the cell membrane (arrowheads), whereas GLUT4 (green) resides in the cytoplasm (double-arrowheads). (b) GLUT3 (red) is present along the cell membrane (arrowheads), whereas GLUT4 (green) resides in the cytoplasm (double-arrowheads). (c) GLUT5 (red) is present along the cell membrane (arrowheads), whereas GLUT4 (green) is in the cytoplasm (double-arrowheads). (d) GLUT1 (green) is present along the sinusoidal membrane (arrowheads). GLUT3 (red) is present along the whole cell membrane with a tendency to concentrate at the canicular domain (arrow). GLUT5 (green) is present along the sinusoidal membrane (arrowheads). (e) GLUT1 (green) is present along the whole cell membrane with a tendency to concentrate at the canicular domain (arrow). (f) A schema showing the localization of sugar transporters (shown in green) expressed in the hepatocytes in the liver. Endogenous GLUT2 is localized at the sinusoidal domain. GLUT1 is also at the sinusoidal domain. GLUT3 and GLUT5 are along the whole aspects of the plasma membrane with a tendency to concentrate at the canicular domain. SGLT1 is restricted to the canicular domain.
Differences in the localization of GLUT5 and GLUT3 in cultured epithelial cells and hepatocytes in situ may reflect the differences in these targeting pathways of apical proteins. GLUT3 and GLUT5 may be delivered initially to the sinusoidal membrane and transcytosed to the canalicular membrane. The apparent distribution of GLUT5 and GLUT3 along the whole membrane may reflect the process of the transport from the sinusoidal membrane to the canalicular membrane. SGLT1, an apical membrane protein in intestinal and kidney epithelial cells and MDCK cells, was exclusively localized to the canalicular membrane in hepatocytes as well. The apparent differences in the localization in hepatocytes in situ among apical proteins SGLT1, GLUT3, and GLUT5 may reflect possible differences in the sorting, targeting, and/or retention mechanism serving these molecules.

**Introduction of exogenous genes into liver by in vivo electroporation and particle gun methods**

Plasmid DNAs can be directly introduced into cells of target organs for local in situ expression by in vivo electroporation [20]. In the preceding study, we introduced GFP gene into the liver of living rats in situ by the in vivo electroporation method [39]. We optimized the condition of DNA injection and electroporation by monitoring the GFP fluorescence in the liver slices. We showed in this work that genes of all sugar transporters tested, GLUT1, GLUT3, GLUT4, GLUT5, and SGLT1, were successfully introduced and expressed in the liver. The localization of these products could be observed immunohistochemically in detail.

We also tested the particle gun methods. In this method, microprojectiles with a diameter of approximately 0.5–2.0 μm, on which plasmid DNAs are attached, are directly introduced into living cells by high-pressure gas. Recently, a hand-held particle gun that does not require a decompression chamber was introduced, making the direct gene transfer into living animal organs much easier [36, 56]. Although the particle gun method is easier with the entire procedure carried out by a single person, and consumes only a small amount of DNA, the successful expression of exogenous genes by this method was limited to 1–3 cell layers of hepatocytes under the connective tissue capsule.

Various kinds of methods have been devised for the introduction and expression of exogenous genes in animal cells in situ [9, 20, 28]. Each method has its inherent advantages and shortcomings. It is, therefore, important to select the method best suited to the specific purpose. Exogenous genes can be expressed by the classical transgenic animal models, which is time- and labor-consuming. Viral vectors have been used [20, 28], but there is a possible risk of infection and carcinogenesis as well as unwanted immune responses. In addition, the presence of receptors for viruses in the target cells is a prerequisite for successful transfection. Compared with methods using viral vectors, in vivo electroporation has several advantages as follows: the technique for transfection of genes is simple and easy; there is no limitation for sizes of transferable genes; it can be used irrespective of species and organs. In addition, successful cotransfection showed that multiple species of genes can be introduced simultaneously into the same cells in vivo. The particle gun method also has similar advantages, although it can be applied only to the exposed surface cells. Further tuning in parameters of electroporation and particle-gun delivery will improve the efficiency of gene expression with minimum cellular damage, making this method a versatile one for in vivo gene introduction.

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**VI. References**


