Chaperoning Organic Anion Transporters Through the Hepatocyte

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Transporters Can Traffic between the Cell Surface and Intracellular Vesicles

How does this movement occur? What are its consequences?
Transporters Inside the Cell Will Not Mediate Ligand Uptake

This suggests an important regulatory mechanism.
Localization of oatp1a1 in Rat Liver by Confocal Microscopy

Red = oatp1a1
Green = actin
(bile canaliculi)
Oatp1a1 Has a PDZ Binding Motif
PDZ Proteins

• Bind proteins having a PDZ binding motif (a 4 amino acid C-terminus consensus sequence)

• There are many PDZ proteins and no way to predict which if any will bind a protein with a PDZ binding motif

• Acronym of the first three PDZ proteins identified:
  - the postsynaptic protein **PSD-95/SAP90**
  - the Drosophila septate junction protein **Discs-large**
  - the tight junction protein **ZO-1**
Binding of a Peptide Ligand to the PDZ-3 Domain of PSD-95

Hypothesis

Oatp1a1 subcellular distribution is specified by its PDZ binding motif
Affinity Chromatography of Rat Liver Lysate Using Oatp1a1 Terminal Peptide

Coomassie

Silver
MALDI Identification of the 70 kDa Band as PDZK1
Western Blots Following Immunoprecipitation of Oatp1a1 from Rat Liver

WB: oatp1a1

PDZK1

oatp1a4

SR-BI
<table>
<thead>
<tr>
<th></th>
<th>PDZK1 (+/+)</th>
<th>PDZK1 (-/-)</th>
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<tr>
<td><strong>OATP1</strong></td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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<tr>
<td><strong>ASGPR</strong></td>
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Plasma Disappearance of $^{35}$S-BSP is Reduced in PDZK1 K/O Mice
Ongoing Investigation

Does oatp1a1 phosphorylation regulate its interaction with PDZK1 and consequently its subcellular distribution and transport activity?

PDZK1 has 4 independent binding sites. What are the other proteins in the PDZK1/oatp1a1 complex?

Oatp1a1 and PDZK1 colocalize to intracellular vesicles. Do these vesicles move on microtubules?
Ntcp Trafficks between the Cell Surface and Intracellular Vesicles

ntcp on the cell surface

$\uparrow$ cAMP

recruitment

? retrieval

ntcp in intracellular vesicles

How does this movement occur?
Do Microtubules Serve as “Railroad Tracks” for Vesicles?

ATP-Dependent Motor Molecules
Motility and Fission of Fluorescent Vesicles on Microtubules After ATP Addition
Ntcp is Present in Endocytic Vesicle Preparations
Movement of Ntcp-Containing Vesicles on Microtubules

![Image of vesicles on microtubules]

Bar graph showing the percentage of motile vesicles in buffer versus 50 μM ATP. The bar for 50 μM ATP is significantly higher than that for buffer, indicated by the number (861) above it.
Colocalization of MT-Based Motors with Ntcp-Containing Vesicles

Percentage of Ntcp-containing vesicles

- Dynein: (1840)
- Kinesin-1 (Kif5B): (1190)
- Kinesin-2 (Kif3A): (855)
- Mouse IgG: (351)
Is Motility of NTCP-Containing Vesicles Regulated?

Previous studies in cells suggested a role for phosphorylation (PKCζ).
Pκζ Is Present in the Rat Liver Vesicles and Colocalizes With Nearly 75% of Ntcp-containing Vesicles
PKCζ Pseudosubstrate Inhibits Motility of Ntcp-containing Vesicles

![Graph showing the inhibition of motility by PKCζ pseudosubstrate. The x-axis represents [PKCζPS] (µM) and the y-axis represents motility (% of total MT-bound). The graph shows a decrease in motility as [PKCζPS] increases.]
PKCζPS inhibits Motility of Early but Not Late Endocytic Vesicles

![Graph showing the effect of PKCζPS on motility of early and late endocytic vesicles. The graph compares the percentage of motile vesicles between Buffer and 50μM PKCζPS in both early and late vesicle populations.](image-url)
Movement of Ntcp Vesicles in HuH7 Cells Expressing GFP-Ntcp
A Cell Permeant PKCζ Pseudosubstrate Inhibits Motility of GFP-Ntcp but not Late Endocytic Vesicles in HuH7 Cells

![Graph showing inhibition of motility by Myr-PKCζPS](image)
Ongoing Investigation

What is (are) the substrates for PKCζ?

How does this regulate motility of these vesicles?

These vesicles also interact with and move on actin microfilaments.
Conclusions

• Uptake of drugs by the hepatocyte is a complex regulated process.

• Understanding the cell biology of transporter trafficking may have important implications for inter-individual differences in drug transport and metabolism.
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