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JB Review
Molecular mechanism of polarized transport

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Mechanisms of generation and maintenance of cell polarity have been investigated using various organisms and cell lines. During and after the establishment of cell polarity, polarized (vesicular) transport as well as cell–cell adhesion is essential. Here, I introduce each molecular step of polarized transport and the molecules involved there. Usually, epithelial cells and neurons are two well-known examples of polarized cells. Thus, I next describe the similarity and difference in polarized transport between these two cell types. Though closely connected, the relationship between cell–cell adhesion and polarized transport remain poorly understood. I will take a few examples indicating the relationship between them. Finally, I will present the future directions in this field.

Mechanisms of generation and maintenance of cell polarity have been investigated mainly using various cell lines, particularly Madin–Darby canine kidney (MDCK) cells (1, 2). To date, though the relationship between cell–cell adhesion and cell polarity has been clarified, the relationship between polarized transport [*i.e.* transport from the *trans*-Golgi network (TGN) to the apical/basolateral plasma membranes] and cell polarity has been elusive. Moreover, a question of whether polarized transport starts after cell–cell contact is established or polarized transport itself is essential for establishment of cell–cell contact is under debate. In addition, the knowledge of molecules involved in polarized transport *in vivo* is still lacking and the search for new molecules is now under investigation.

By use of green fluorescent protein (GFP) technology, we are now able to look at the intracellular transport in living cells. So, I will first describe the live process of polarized transport and then describe each step.

The Route of Intracellular Vesicular Traffic (Polarized and Non-polarized Transport)

Kai Simons' group has described the transport of apical and basolateral transport vesicles and found out the following basic behaviors of vesicles (3). First, in polarized cells as well as in non-polarized cells sorting takes place at the TGN. Secondly, apical and basolateral vesicles are separately transported to their final destinations and fused with apical and basolateral plasma membranes, respectively. Thirdly, basolateral-destined vesicles are not transported through endosomes.

However, Mellman's group showed that vesicles, which transported VSV-G (vesicular stomatitis virus G) protein, a basolateral protein, pinched out from the TGN, and went through the recycling endosomes to the plasma membranes (4). Nowadays, a number of groups come around to that opinion.

In addition, it is known that: (i) apical-destined vesicles carrying raft-dependent cargos and those carrying raft-independent cargos are distinct; and that (ii) basolateral-destined vesicles are divided into several groups according to their cargos.

Steps in polarized transport and the proteins known to be involved in each step

Molecules involved in each step are depicted in Fig. 1.

Sorting
It is known that the sorting between apical and basolateral transport is carried out basically at the TGN. The sorted vesicles are transported by distinct vesicles. Thus, many researchers believe that some molecules crucial for sorting are localized at the TGN.

Signals and molecules for basolateral sorting. Several sorting signals for basolateral membranes are known, such as those containing tyrosine motif and those containing dileucine motif (5). Adaptor proteins known as AP-1B and AP-4 are known to bind these motifs and to be involved in basolateral sorting (6, 7). In addition, clathrin itself has recently been shown to be essential for basolateral sorting (8). Protein kinase D2 (PKD2), a kinase that binds diacylglycerol (DAG), has also been shown to be necessary for fission of basolateral vesicles (9).
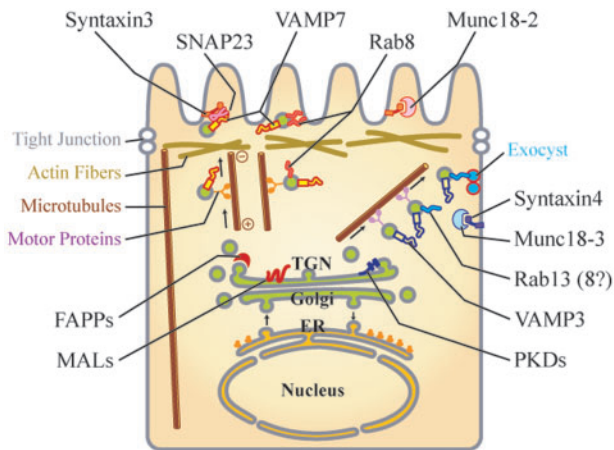
Fig. 1 **Molecules involved in polarized transport.** Molecules involved in apical transport are depicted in red and those involved in basolateral transport are depicted in blue.

Fig. 2 **Phenotype of Rab8a knockout mouse.** Apical marker DPPIV (white staining) is localized on the plasma membrane in wild-type mouse (upper panel), whereas in Rab8a knockout mouse, most of DPPIV is localized in large granular structures (lower panel).

Signals and molecules for apical sorting. Apical signals: GPI signals and glycosylation (N- and O-glycosylation) are known to be important in apical sorting (*10*). However, even proteins with these signals are transported basolaterally in the presence of basolateral signals within their cytoplasmic domains. From these findings and the observation that some amino acids in the transmembrane region are essential for apical transport, 'raft hypothesis' is now prevailing (*11*). 'Rafts' are also known as glycolipid and cholesterol containing membrane domains where GPI-anchor proteins and glycosylated proteins are accumulated. However, there are many apical proteins which do not associate with rafts. Thus, apical transport is also mediated by several mechanisms. Jacob's group has been investigating this point for several years (*12*, *13*). They labelled sucrose–isomaltase (SI) and lactase–phlorizin hydrolase (LPH) with YFP (yellow fluorescent protein) and CFP (cyan fluorescent protein), respectively, and showed that the vesicles they reside in are transported differently. They purified these vesicles and showed that the vesicles containing SI also have annexin2, and that those with LPH have galectin3. They further analysed galectin3 knockout mice and found that LPH, which is normally localized at the apical plasma membrane, is diffusely distributed in the small intestinal cells (*14*). From these findings, formation and sorting of vesicles carrying raft-independent cargos are mediated by crosslinking of luminal sugars with galectin3.

Previously, proteolipids, such as MAL and MAL2, were candidates of sorting proteins of apical vesicles (*15*). However, recent study showed that MAL knockout mice failed to show overt neuronal and epithelial polarity defects (*16*). Thus, MAL deficiency is not sufficient to exert abnormality in apical transport. Other groups reported that FAPP2, a PH domain containing protein, which binds Arf and PIP2, is involved in apical sorting (*17*).
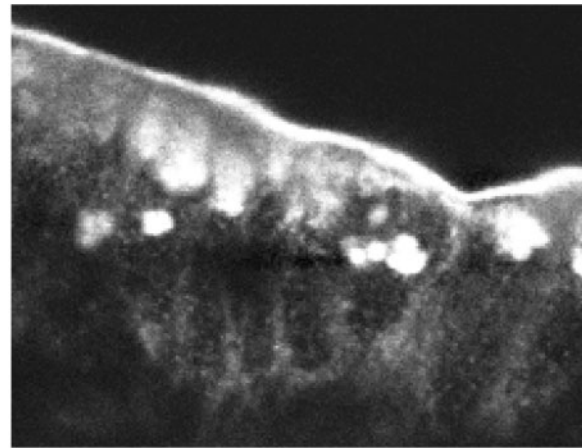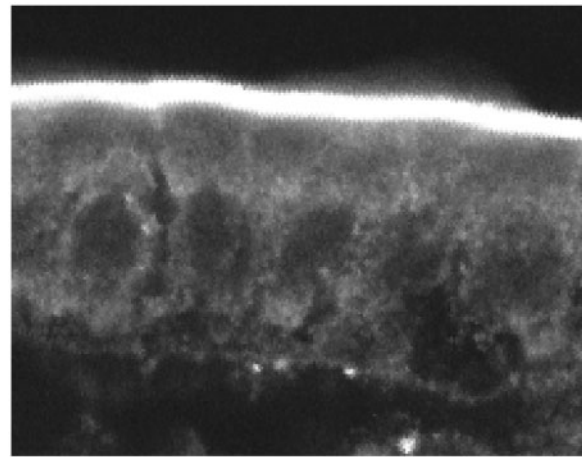
Transport

Motor proteins are absolutely required for transport from the TGN to the plasma membranes. It is believed that for the long distance transport, microtubule-mediated transport by kinesins and dyneins are necessary and for the short distance transport, actin-mediated transport by myosins is necessary (*18*).

Thus, to understand the transport in polarized cells, information on polarity of microtubules is absolutely required. Minus and plus ends of microtubules are directed to apical and basolateral plasma membranes, respectively. Thus, apical transport has long been thought to be mediated through minus-end directed motor proteins, such as cytoplasmic dynein (*19*) and KIFC3 (*20*). However, recent finding indicates that the directions of microtubules are mixed below the apical plasma membrane. Furthermore, KIF5B, one of conventional kinesins, carries vesicles containing p75NTR, a raft-independent cargo molecule (*21*). From these findings, distinct motor proteins are used to convey distinct cargo proteins. In addition, Astanina and Jacob have shown that KIF5C is involved in apical transport in MDCK cells (*22*).
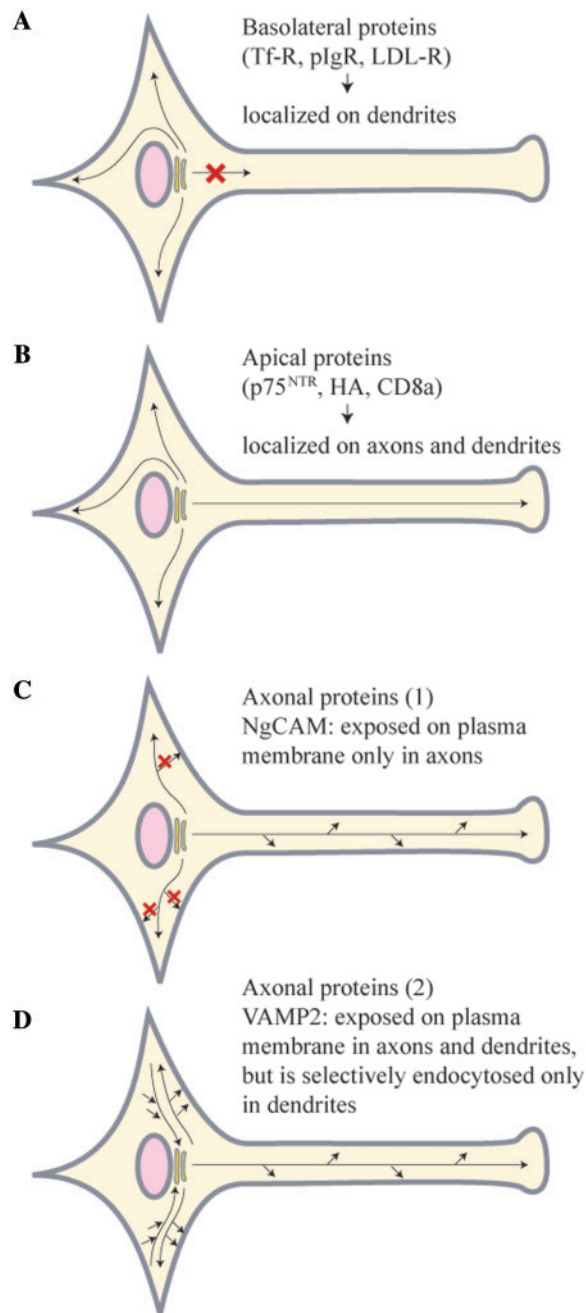
Fig. 3 Routes of 'apical', 'basolateral' and 'axonal' proteins in the neuron. (**A**) 'Basolateral' proteins (Tf-R, pIgR, LDL-R) are transported to the dendrites directly and are exposed on the dendrite membranes. (**B**) 'Apical' proteins (p75NTR, HA, CD8a) are transported both to axons and dendrites and exposed on axonal and dendritic plasma membranes. (**C**) One 'axonal' protein (NgCAM) is transported both to axons and dendrites, but is exposed only on the axonal plasma membranes. (**D**) The other 'axonal' protein (VAMP2) is transported also both axons and dendrites, but is selectively endocytosed only in dendrites.

In case of neurons, plus ends of microtubules are localized at the distal ends of axons. This is in sharp contrast to the epithelial cells where minus ends of microtubules are localized near the apical plasma membranes. Thus, transport from the cell body to the synaptic terminal is mediated by kinesin family motor proteins and the transport in the opposite direction is mediated by cytoplasmic dynein. What makes things more complex is that in dendrites, the microtubules are of mixed polarity, with some plus ends pointing outward and some pointing inward (23).

Tethering

Basolateral tethering. Until recently, a basolateral tethering factor on the transport vesicles has been thought to be Rab8 and tethering factors on the basolateral plasma membranes has been thought to be components of exocyst (24). However, our group revealed that Rab8 is essential for localization of apical proteins in the small intestine (25) (Fig. 2). Since other groups have also shown that Rab8 is necessary for the generation of cilia which protrudes from the apical plasma membranes (26–28), it is becoming popular to think that Rab8 is necessary for apical transport, although the exact role of Rab8 (for example, whether it is required for sorting or tethering) is unclear.

Apical tethering. Annexin13b was shown to be necessary for apical tethering (29), but further molecular details have not been shown.

Fusion

For fusion of vesicles to the plasma membranes, it is widely accepted that SNAP (soluble NSF attachment protein) receptors (SNARE) proteins and SNARE-binding proteins are thought to be important. For apical transport, syntaxin3 and SNAP23 are t-SNAREs and VAMP7 (TI-VAMP) is a v-SNARE. For basolateral transport, syntaxin4 is a t-SNARE and VAMP3 is a v-SNARE (30). In case of VAMP7, a dominant negative form suppressed axonal elongation (31). Knockdown of syntaxin3 also inhibited axonal elongation (32).

Direct or indirect pathway?

It is known that there are at least two steps to the final destinations (e.g. apical membrane). One is direct pathway, the other is indirect pathway.

The former is the pathway where the cargo is transported directly to the final destination. The latter is the one where the cargo is transported to the different sites and then endocytosed vesicles are redirected to the final destination. The rate of pathways a cell uses is different between the species of the cell. For example, the tubular epithelial cells in the kidney are believed to use mainly the direct pathway, whereas hepatocytes are believed to use the indirect pathway. Intestinal epithelial cells are believed to use both (33).

Differences in polarized transport between epithelial cells and neurons

Epithelial cells and neurons are two typical types of polarized cells. It was believed that the axons and dendrites correspond to the apical and basolateral plasma membranes, respectively. However, current investigations showed that this model is an oversimplified one. In case of neurons, Banker's group has shown as follows; (i) basolateral proteins (e.g. Tf-R) are localized
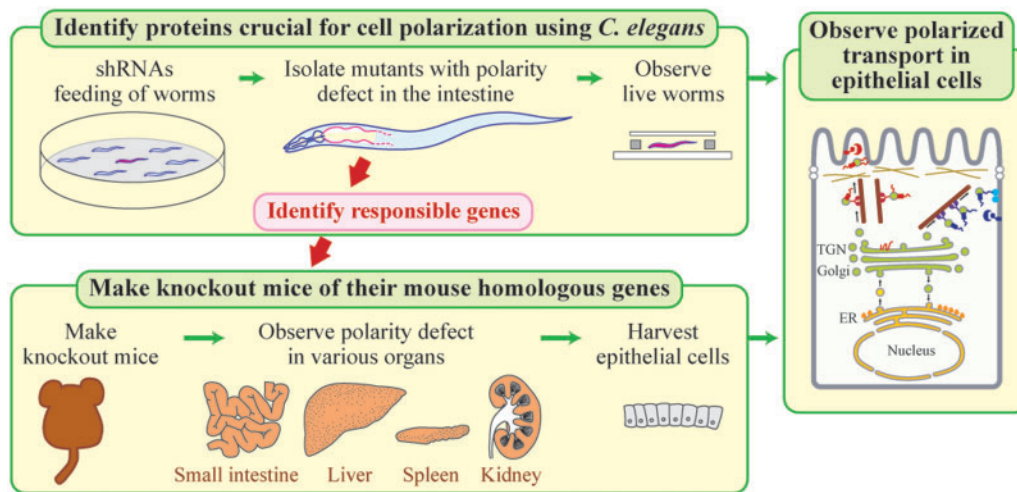
Fig. 4 One of the future directions in the research of cell polarity using multiple organisms. After finding out novel cell polarity-related genes using simple organisms, such as *Caenorhabditis elegans*, we will be able to knock out the genes in mice and/or track the vesicular transport in live worm or mouse cells (from wild-type or knockout animals) under light microscope.

on dendrites, but apical proteins (e.g. p75NTR) are localized both on axons and dendrites; (ii) dendritic membrane proteins are transported directly to the plasma membranes; (iii) axonal proteins (VAMP2 and NgCAM) go to the axons and dendrites: NgCAM is exposed on the plasma membrane only in the axons, while VAMP2 is exposed on the plasma membrane both in the axons and dendrites, but is selectively endocytosed only in the dendrites (34) (Fig. 3). However, different groups have reported other routes of transport (35).

In addition, it is doubtful that epithelial cells and neurons use the similar mechanism or proteins in their polarized transport because many proteins involved in polarized transport show different pattern of tissue expression. Thus, it is feasible to think that some proteins are important in a certain type of epithelial or neuronal tissues. For example, our Rab8 knockout mice showed polarity defects mainly in the epithelial cells of the small intestine (25), showing the limitation of using cell culture as a general model system for polarity research.

Interaction between cell–cell contact and polarized transport at the molecular level

Until recently, it is generally imagined that first cell–cell contact makes the apical and basolateral regions and then, apical and basolateral proteins are transported by polarized transport. However, accumulating evidence indicates that differentiation of the apical and basolateral regions can take place without cell–cell contact.

One of the first and striking observations is that if a molecule, called Strad, is overexpressed, cells without cell–cell contact are able to have apical microvilli (36). Secondly, epithelial cells without ZO-1 and ZO-2 do not form tight junction, but still they have the apical and basolateral regions (37). These results showed that the polarized transport is essential for the generation and maintenance of cell polarity as well as cell–cell contact. Thus, in normal polarization process, it is

natural to think about close interaction between cell–cell contact and polarized transport.

However, very few observations have been reported about interaction between cell–cell contact and polarized transport so far. One of them is the observation in Dlg5 knockout mice (38). These knockout mice showed cystic kidney from polarity defect of renal tubules. Normally, vesicles carrying cadherins and catenins use vesicular Dlg5 to fuse with the plasma membranes through the interaction between Dlg5 and syntaxin4 on the basolateral plasma membrane. In the renal tubules of knockout mice, this interaction was perturbed because of lack of Dlg5, resulted in transport defects of cadherins and catenins to the basolateral plasma membrane.

Future directions

In this review, I introduced molecules involved in polarized transport whose roles have mainly been revealed in cell culture system, particularly MDCK cells. However, their roles in tissues and functions in organisms have not been clarified. To elucidate these, we are now making knockout mice of many of these molecules. As a first report, we showed that Rab8 is essential for localization of apical proteins in the epithelial cells of small intestine, which is in sharp contrast to previous reports (25). In addition to this, we speculate that much more molecules should be involved in the generation and maintenance of cell polarity. Thus, finding out of novel molecules and investigating the roles of these molecules should be the next task to fully understand the molecular mechanism of cell polarity (Fig. 4). As cell polarity is essential for building the tissue and its function, the defects of polarity should lead to a number of diseases, such as cancer and metabolic syndrome. Since further investigation should lead to treatment of these diseases, the necessity of cell polarity research is still increasing.

Conflict of interest
None declared.

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