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REVIEW

The Role of Motor Proteins in Signal Propagation

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Abstract—The signaling and transport systems of eucaryotic cells are tightly interconnected: intracellular transport along microtubules and microfilaments is required to position signaling-pathway components, while signaling molecules control activity of motor proteins and their interaction with tracks and cargoes. Recent data, however, give evidence that active transport is engaged in signaling as a means of signal transduction. This review focuses on this specific aspect of the interaction of two systems.

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In the fourth edition of the remarkable textbook "Molecular Biology of the Cell", in the chapter focused on the organization of signaling complexes, one can read the following: "The scaffold strategy provides precision, helps to create a large change in MAP-kinase activity in response to small changes in signal molecule concentration, and avoids cross-talk. However, it reduces the opportunities for amplification and spreading of the signal to different parts of the cell, which require at least some components to be diffusible" [1]. Since the time it was written some facts came to light that suggest that the cell could use active transport by motor proteins as a much more effective means than simple diffusion to propagate a signal in the form of multicomponent complexes. This review considers such facts that have been discovered mostly during the last 15 years.

Generally speaking, the dependence of signal transduction on intracellular transport is obvious. Indeed, receptors of extracellular ligands synthesized in endoplasmic reticulum are delivered to the plasma membrane in membranous vesicles, after ligand binding they are internalized, and, if not subjected to degradation, are then restored and return to the plasma membrane. In addition, extracellular signal molecules also are delivered to the plasma membrane and exit from the producing cell into the intercellular space by exocytosis. These processes required for maintenance of the signaling system in operative condition are served by the transport provided by motor proteins.

The transport system of the cell is based on the use as tracks of two kinds of cytoskeletal structures: microtubules and microfilaments – polar polymers with differing ends. Three classes of motor proteins – large mechanochemical ATPases that can transform energy of ATP hydrolysis into mechanical movement – move along these tracks. Kinesins that move cargo mostly to the plus ends (anterogradely) and dyneins, in the opposite direction (that is, moving retrogradely), are associated with microtubules, while myosins translocate their cargo mostly toward the plus ends of actin filaments [2-4]. Some motor proteins use various mechanisms to bind different cargoes to carry them along a track. Several proteins have been identified that provide connection between various motor proteins and specific cargoes. It is remarkable that such adaptors appear often as proteins that were known before, for example, the so-called scaffold proteins organizing multicomponent signaling complexes [5] – this fact indicating the close connection of the transport and signaling systems. I shall show one example that clearly demonstrates the role of intracellular transport in organization of signaling pathways.

In *Caenorhabditis elegans*, during development of the vulva, epithelial precursor cells receive a signal from a special anchor cell in the stroma that secrets EGF. This factor must bind to the corresponding receptor tyrosine kinase LET-23 and trigger differentiation and proliferation of the vulva cells through activation of the Ras/MAP-kinase signal cascade. Because the anchor cell is situated in the stroma, while EGF is unable to come through tight junctions between epithelial cells, such receptors must be located within the basolateral domain of the plasma membrane of precursor cells. Analysis of *C.*

elegans mutants revealed three genes – *Lin2*, *Lin7*, and *Lin10* – whose products are necessary for such localization and interact by their PDZ domains to form a complex that binds LET-23 [6]. For any of these genes, a mutant embryo was lacking a normal vulva because of incorrect localization of LET-23 on the plasma membrane. This work provided the first experimental evidence suggesting that signaling molecules have to be not only present but properly localized within a cell. Such localization can be provided only by the directed transport, although it is unclear what motors perform the transport in this specific situation. Some light has been shed by the study of the functions of the same proteins in nerve cells. They turned out to be involved in neuronal transport and recycling of NMDA receptors, not only in *C. elegans*, but in mammals also. This kind of transport is performed by kinesin KIF17 – a specific partner of Lin10 [7]. As was disclosed later, the retrograde transport of AMPA receptors in recycling endosomes depends on Lin10 as well [8].

So, positioning of the receptor on the plasma membrane and its recycling really depend on intracellular transport. However, is active transport required, in the narrow sense, for signal propagation? Provided this is the case, this phenomenon should necessarily be very complicated, because the motor protein activity in the cell is regulated by various mechanisms, and some of them involve signaling molecules. In other words, a "carrier" might be controlled by a cargo it carries.

There are many signaling pathways in the eukaryotic cell that are organized on the basis of scaffold proteins, and this principle might explain a remarkable property of the signaling system: from the same enzymes different cells have built different independent pathways responding to different stimuli and producing different reactions. Classical examples of such organization are signaling complexes of Mitogen-Activated Protein Kinases [9]. Three types of serine/threonine protein kinases: MAP-kinase (MAPK), MAP-kinase kinase (MAPKK), and MAP-kinase kinase kinase (MAPKKK) are components of the signaling cascades activated by mitogens. Kinases join into complexes in various combinations, while specificity of these signaling modules is supported by scaffold proteins.

A typical example of the signaling cascade that employs both scaffold and motor proteins is the c-Jun NH_2-terminal kinase (JNK) pathway. This is a MAPK activating a number of transcription factors, among others the factor c-Jun. Mammals have three genes encoding JNKs: *MAPK8*, *MAPK9*, and *MAPK10*; from their protein products, JNK1 and JNK2 are expressed ubiquitously, while JNK3 mostly in nervous tissue. JNKs become activated in mammalian cells in response to stress conditions or the action of inflammatory cytokines. The signaling modules in JNK-connected signaling are organized by using scaffold proteins JIPs (JNK-Interacting-Proteins), which differ in their functions and, by binding to different MAPKKK, MAPKK, and MAPK, provide different pathways [10]. Thus, JIP-1 collects the signaling module MLK–MKK7–JNK1 [11], while JIP3 collects MEKK1–SEK1–JNK3 [12]. From four known today members of this scaffold-protein family, JIP1, JIP2, and JIP3 take part in activation of JNK-signaling, while later found JIP4 is involved into signal transduction to another MAPK, p38, through specific MAPKKs – MKK3 and MKK6 [13].

All four JIPs can bind kinesin-1 through its light chain [14, 15]. Kinesin-1 is a ubiquitously expressed motor protein, a member of the kinesin superfamily, which is able to effectively transport along microtubules a variety of cargoes. Its molecule is composed of two parallel heavy chains containing motor domains and two light chains, which are located at the end of the molecule opposite to the motor domains. The light chains are involved in cargo binding through their specific TPR domains that provide protein–protein interactions. JIPs have been identified as partners of kinesin-1 by using two-hybrid analysis with the light chain of kinesin-1 as bait [14, 15]. Do they, however, mediate kinesin-dependent transport of JIP-organized complexes along microtubules? Or could this interaction indicate solely that kinesin-1 is regulated via phosphorylation by JIP-associated kinases?

This question was answered by studies of axonal transport – a complicated phenomenon that stipulates functioning of neurons because the extremely polarized shape of neurons excludes a significant role for diffusion. All three JNKs function in neurons; JNK1 being considered constitutively active and JNK2 and JNK3 being activated in response to stress. In particular, JNK3, on one hand, participates in stress-induced apoptosis of nerve cells [16]; on the other hand, it regulates regeneration of peripheral nerves [17]. JNKs are transported along the axon in both directions [18]; however, expression of additional recombinant JNKs differently affects fast axonal transport: JNK1 does not induce any changes, JNK2 partially inhibits anterograde transport of some cargoes, and JNK3 depresses both directions of transport [19]. It is not yet clear what stipulates this difference and what the mechanism of such inhibition of the transport function is; however, these facts clearly indicate that signaling molecules indeed control the activity of motor proteins.

At the same time, the special study on CAD cells, which in culture develop neurite-like outgrowths, proved that JIP1 behaves as a real cargo and is transported by kinesin-1: exogenous JIP1 was concentrated in the outgrowth tips, at the plus ends of microtubules. This localization was dependent on the light chain of kinesin-1, indicating the active role of this motor [15]. Besides kinesin-1, JIP1 was found to bind to regulatory kinase DLK (Dual Leucine Kinase, which is a kinase of the MAPKKK "level") and the transmembrane Reelin receptor ApoER2 [15]. Thus, although the presence of

some other components in this transport complex cannot be excluded, identification of these partners suggests that JIP1 binds together three kinases of the MAP-kinase pathway, transmembrane receptor that connects the complex to membrane vesicles and kinesin-1 capable of transporting the whole complex along microtubules toward their plus ends [15].

The CAD cells are some kind of neuron analogs; it is therefore possible to suggest that the same transport occurs in axons. However, what is the role of the transport of signaling kinases from the cell body toward the end of the outgrowth? Activated MAP-kinases take part in transduction of the signal from the cell surface to the nucleus, this direction bearing no relation to kinesin. In what form are such complexes transported? Where can such a complex be combined and where does kinesin-1 carry it?

One suggestion: kinesin provides neurogenesis [15], that is, growth of neurites and their branching. Then we should suggest that the complex of signaling kinases is delivered to the position where it will be able to accept the signal and pass it to the nucleus. Indeed, association of MAPK complexes with ApoER2-bearing membrane vesicles might indicate that upon transport termination this complex becomes located close to the plasma membrane. Along with this, analysis of *Drosophila* mutants by individual components of the MAP-kinase complex – Wallenda, or wnd (corresponds to DLK, that is MAPKKK), Hemipterous, or hep (corresponds to MKK7, that is MAPKK) and Basket, or bsk (corresponds to JNK, that is MAPK) – revealed that each is required for normal axonal transport. Indeed, accumulation of synaptic proteins was observed in the nerves of flies mutant in any of these proteins [19]. Notably, the *Drosophila* homolog of JIP1, APLIP1 (Amyloid Precursor Protein-Like Interacting Protein 1), does not contain a JNK-binding domain [20], but it is able to bind upstream kinase hep and kinesin-1 light chain. Co-immunoprecipitations showed that phosphorylation-activated kinase hep induces dissociation of APLIP1 from kinesin light chain (or prevents their interaction) [19]. Whether analogous to JNK kinase bsk takes part in this effect remained unclear, but whatever the mechanism is, the breakdown of connection between light chain and APLIP1 as a consequence of MAP-kinase activation should release kinesin and thus designate the terminal point of the transport.

Interestingly, the transport of synaptobrevin-carrying secretory vesicles, with which APLIP1 is associated in axons, in APLIP1 mutants of *Drosophila* embryos slows down in both directions [21]. Does this mean that the scaffold proteins of this type are able to interact with oppositely directed motors?

The positive answer to this question was obtained upon examination of axonal transport of APP (Amyloid Precursor Protein) itself – the integral membrane protein notorious because of its role in progression of Alzheimer's disease. Normal APP is actively transported along axons in both directions with accumulation close to synapses. Its function is not quite clear, but it is suggested to take part in synapse formation and reparation after injury. APP is also known to interact with Reelin, this interaction supporting neurite growth [22]. Reelin, the receptor of which ApoER2 is incorporated into the transport complexes through its interaction with JIP1 [15], is a glycoprotein secreted into the intracellular space that has multiple functions in brain. In particular, it regulates APP processing, disruption of which causes neurodegeneration. APP is located at membrane vesicles bearing both kinesin-1 and dynein; movement of these vesicles is highly processive with long fast runs in both directions [23].

Recent studies showed that JIP1 coordinates kinesin-1 and dynein activity in APP trafficking: JIP1 knockdown inhibited both directions of this transport. Coordination is based on phosphorylation of participants of the process. APP itself is able to bind the light chain of kinesin-1 [24] independently of whether APP is phosphorylated or not; however, it interacts with JIP1 only being phosphorylated at threonine 668 [25]. By the way, the scaffold protein APLIP1 was also so-named: APP-Like Interacting Protein 1 – due to interaction with the APP homolog in *Drosophila* [19]. It is noteworthy that APP is phosphorylated by JNK; however, it requires JIP3, but not JIP1, for this to occur [25]. Depletion of JIP1 in the cell using RNA interference depresses transport of only the phosphorylated fraction of APP. It appears that in any case APP can be translocated by kinesin-1, but if it is phosphorylated its transport depends on JIP1.

All these data concern anterograde axonal transport; the opposite direction of APP transport is served by dynein and its cofactor dynactin, a multicomponent complex that regulates dynein work and mediates dynein binding to a variety of cargoes [26]. JIP1 proved to interact with dynactin subunit p150^{Glued}, and this interaction led to competitive inhibition of kinesin-1 activation *in vitro* and disturbance of APP transport in neurons [23]. Kinesin-1 and p150^{Glued} compete with each other for binding to JIP1; accelerated anterograde transport of APP is observed upon expression of mutant phosphomimetic JIP1 (S421D) [25]. Fu and Holzbaur [23] propose a model that considers all data on the transport of APP in axons: activated JNK phosphorylates APP, which then binds JIP1; the direction of transport of this complex depends on whether JIP1 is phosphorylated itself, because when phosphorylated, it prefers kinesin-1. According to this model, phosphorylation of JIP1 at serine 421 by JNK switches direction of APP translocation [23]. It is not clear, however, in what cases such switching is required and whether it is accompanied by any change in the composition of the transport complex.

Activation of JNK mediates inhibition of axonal transport by huntingtin (Htt), a protein involved in neurodegeneration in Huntington's disease. Htt is a scaffold protein organizing some cargoes for their movement

along microtubules. Mutant pathogenic Htt, modified by addition of several CAG triplets to the coding sequence and, as a consequence, lengthening of the polyglutamate region in its polypeptide chain (poly-Q-Htt), depressed fast axonal transport although Htt itself does not interact with motor proteins. A certain role in this effect can be played by an associated with Htt protein HAP1 (Huntingtin-Associated Protein 1) [27]. HAP1 appears to promote transport along microtubules in both directions, because the light chains of kinesin-1, as well as dynactin subunit p150^{Glued}, have been identified as its partners [28, 29]. Both HAP1 and Htt are known to be involved into certain steps of axonal transport of endosomes, lysosomes, autophagosomes, and mitochondria; these kinds of transport are disturbed in the absence of Htt or in the presence of its mutant variants [30-32].

There is, however, one more interesting putative mechanism of inhibition of axonal transport by mutant Htt. Poly-Q-Htt induces specific activation of JNK3 (but not JNK1/2), and activated JNK3 phosphorylates the motor domain of kinesin-1 at serine 176; this modification significantly weakens kinesin-1 binding to microtubules [33]. It is noteworthy that phosphorylation of this amino acid residue within the motor domain, which is highly conservative and critical for kinesin-1 function, is not a trouble but rather a normal way of kinesin-1 regulation [33]. Therefore, normally, and not only in Huntington's disease, activation of JNK3 within a transported signaling complex must disturb the kinesin-1 interaction with microtubules and interrupt the transport at some distinct point where this activation will occur.

As already mentioned, MAP-kinase JNK3 fulfills specific functions in neurons by participating in reparation of injured nerve outgrowths and controlling stress-induced apoptosis of nerve cells. To repair the injured outgrowth, transcription of some genes must be renewed, which suggests signaling from the site of injury to the nucleus. In this case signal transduction clearly occurs via active transport in the retrograde direction.

As revealed during observation of axonal transport in live *Danio* fish, at least two various cargoes are transported retrogradely using JIP3: activated JNK3 and lysosomes [34]. JNK3 is activated upon injury, but the presence of activated JNK3 causes defects in the structure of the axon terminal; thus, the fast transport might be required for removing the active kinase from the dangerous zone. Interestingly, the transport of lysosomes, which is also served by JIP3, occurs independently of JNK3 [34].

The transcription factor c-Jun that is activated by the JNK signaling cascade plays an important role in axon regeneration, and, therefore, JIP3-dependent transport of JNK3 might contribute to regeneration through c-Jun activation [35, 36]. The signal of injury is delivered to nucleus by retrograde transport provided by the interaction of JIP3 with dynactin. The phase of signaling about injury implicating dynein is followed by the phase of growth renewal supported by kinesins [37].

JIP3 proved to be involved also into a specific kind of retrograde transport in neurons – transport of signals from trophy factors, neurotrophins, which control survival and normal functioning of nerve cells. There are four known neurotrophins: Nerve Growth Factor (NGF), Brain-Derived Growth Factor (BDNF), and neurotrophins 3 and 4. These growth factors play the role of ligands for receptors TrkA, TrkB, TrkC (the so-called Tropomyosin receptor kinases), and p75 receptor in the distal region of the axon [38]; in response, receptors dimerize and phosphorylate each other, this process leading to elevation of the total catalytic kinase activity. Activated Trk tyrosine kinases function in growth and differentiation of nerves through activation of various signal cascades mediated by small GTPases Ras and Rac, phospholipase C, and phosphatidylinositol-3-kinase, which leads to the final activation of corresponding transcription factors controlling transcription of target genes [39].

Dynein is able to bind all three types of Trk receptors via interaction with their cytoplasmic domains by its light chain Tctex-1 and intermediate 74-kDa chain; this fact indicates the possible participation of dynein in retrograde transport of Trk receptors within so-called signaling endosomes containing complexes of neurotrophic factors and their receptors, as well as their effectors [40, 41]. Indeed, observation of fluorescently labeled Trk-receptor trafficking in real time showed that dynein is absolutely required for their transport from axon terminal toward the neuron body after receptor activation and internalization [41]. In the absence of dynein, neurotrophin, though interacting with the axon terminal, is unable to pass the signal for survival to the cell body, and such neurons are subjected to apoptosis [42].

There appears to be a specific immobilization of dynein at various types of Trk receptors. Thus, the dynein adaptor snapin is specific for TrkB and takes part in formation of signaling endosomes on the basis of the receptor TrkB [43]. This receptor accepts the signal from neurotrophin BDNF, which regulates growth and branching of dendrites in cortical neurons. Dynein binds to snapin by its intermediate chain IC-1B that is specific for neuronal tissue [43, 44], and this binding presumably stipulates its recruiting to receptors TrkB. Thus, in this case, again, dynein performs signal transduction from axon terminal to nucleus, and this signaling results in regeneration or lengthening of neuron outgrowths.

However, this kind of retrograde transport is also supplemented by its opposity – anterograde transport of TrkB receptors in axons and dendrites that is fulfilled by kinesin-1 and the scaffold protein JIP3 [45]. Signaling endosomes carrying TrkB receptors bind JIP3, which directly interacts with the cytoplasmic domain of TrkB that consists of 12 amino acid residues and is adjacent to

the plasma membrane [45]. Axonal transport of TrkB that is mediated by JIP3 leads to BDNF-induced activation of signaling kinase Erk. As a result, additional TrkB receptors are recruited to the membrane of the distal segment of the axon to facilitate the BDNF-induced retrograde way of signal propagation [45].

In their recent review, Rishal and Fainzilber suggested a mechanism of coordination of two directions of transport supporting axon regeneration [37]. Their ingenious model of oscillating signal assumes that signal from the neuron body is anterogradely transported by kinesins to the neurite end where it activates dynein-dependent retrograde transport of some another cargoes toward the cell body. Retrograde signal represses initial anterograde signal, that is, the system is periodically "reset". This coordination results in oscillating retrograde signal, the frequency of which goes down as the cell dimensions increase. Such mechanism could explain the origination of the signal of injury simply by axon shortening and corresponding change in oscillation frequency [37].

These putative oscillations resemble the behavior of the so-called shuttle proteins that are involved in regulation of transcription of certain genes in response to external signals. And because no one known shuttle protein possesses motor activity, one could suggest that these rhythmical translocations are connected with functioning of dynein and kinesins, so this is again an example of involvement of the transport system in signaling. The best studied in this context are signal translocations of the Smad protein family (Sma and Mad Related Family).

Smad proteins receive signals from receptors of the growth factors TGF-β/Activin/Nodal, which regulate various processes connected with growth and development. Their receptors are serine/threonine kinases. Upon receiving signal, TGF-β receptor of RII type phosphorylates the TGF-β receptor of RI type and binds it for phosphorylation of Smad2 and Smad3, which then form a complex with Smad4, and the whole complex goes to the nucleus [46]. Interestingly, participants of this signaling pathway are constantly shuttling between the peripheral cytoplasm and nucleus, thus providing a mechanism for constant monitoring of the receptor activity [47, 48]. A broad study using vital fluorescence microscopy that has been carried out on *Xenopus* and *Danio* embryos, as well as cultured mammalian cells, demonstrated that Smad2 shuttles between nucleus and cytoplasm, and this behavior does not depend on whether factors of the TGF-β/Activin/Nodal family are present in the medium [49]. Treatment of cells with Activin induced accumulation of Smad2 within nuclei, and this effect depended on microtubules and kinesin-1, thus indicating that kinesin-1 participates in this signal pathway. In the dephosphorylated state (that supposedly corresponds to the Smad2 state upon exit from the nucleus) Smad2 bound to the light chain of kinesin-1, while the light-chain fragment inhibited Smad2 accumulation within the nucleus in response to the action of Activin. In an attempt to explain this effect, the authors of this study suggest that the function of kinesin-1 is the well-timed delivery of dephosphorylated Smad2 to the cell periphery, where it can again receive a signal from the still active receptor [49].

The retrograde direction of Smads transport from the receptors that activate Smad proteins to the nucleus, where they regulate transcription, occurs presumably by dynein. The elevated expression of a component of dynactin, dynamitin, a condition that has been shown to disturb various dynein-dependent transport processes, prevented Smad2 from entering the nucleus upon stimulation by TGF-β [50]. In addition, Smad2 and Smad3 have been found to bind the same light chain of dynein but its various isoforms: Km23-1 for Smad2 and Km23-2 for Smad3 [50, 51]. It appears that although Smad2 and Smad3 are structurally very similar, they fulfill different functions and, upon receiving signals from TGF-β, take part in different processes, in which different dynein molecules are engaged [51, 52]. The interaction of Smad2 with Km23-1 required its phosphorylation and proved to be necessary for accumulation of phosphorylated Smad2 within the nucleus upon the action of TGF-β [50]. These facts disclose a direct role of dynein in functioning of Smads in transduction of signal from TGF-β.

All the data described in this review give evidence that signaling and transport systems of the eukaryotic cell are tightly interconnected, although the process of their interaction is often too complicated to isolate any specific aspect. In spite of many new facts, there are multiple questions left so far unanswered concerning details and rules of the transport of signaling molecules. In the general case, dyneins are aimed at providing fast signal propagation from the cell surface receptors to the nucleus for switching of gene transcription. The role of kinesins is presumably the constant maintenance of the competent state of the signaling machine.

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