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REVIEW

Dynamics of Nonmembranous Cell Components: Role of Active Transport along Microtubules

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Abstract—Here we discuss some common mechanisms of microtubule-dependent active transport of nonmembranous components in animal cells. We summarize data about mRNA, cytoskeletal elements, structural proteins, and signaling complexes transport. We also characterize the series of molecular interactions that connect nonmembranous cargoes and microtubules and describe the regulatory pathways for these interactions.

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The role of microtubules in transport within cytoplasm of such membrane organelles as mitochondria, Golgi apparatus components, exo- and endocytotic vesicles, etc. is widely known. Numerous reviews deal with this problem as well as with general problems of transport regulation [1-6]. However, there are also nonmembranous cell components whose distribution in the cytoplasm or intracellular motility depends on microtubules. In recent years, an extensive literature has accumulated concerning such cell components, and this has stimulated us to summarize the available data. Some limitations are necessary due to extensive volume of the subject. Here only problems concerned exclusively with transport in interphase cells will be considered, but the other functions of microtubules, in particular their special role in motility of mitotic chromosomes, will still be kept in the background. Besides, we shall consider only examples of nonmembranous component transport in animal cells.

Abbreviations: APC, adenoma polyposis coli protein; CPE, cytoplasmic polyadenylation element; MSD, mean squared displacement; NLS, nuclear localization signal; RNP, ribonucleoprotein; UTR, untranslated region.

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ACTIVE TRANSPORT — ITS LOGIC AND MOTOR PROTEINS

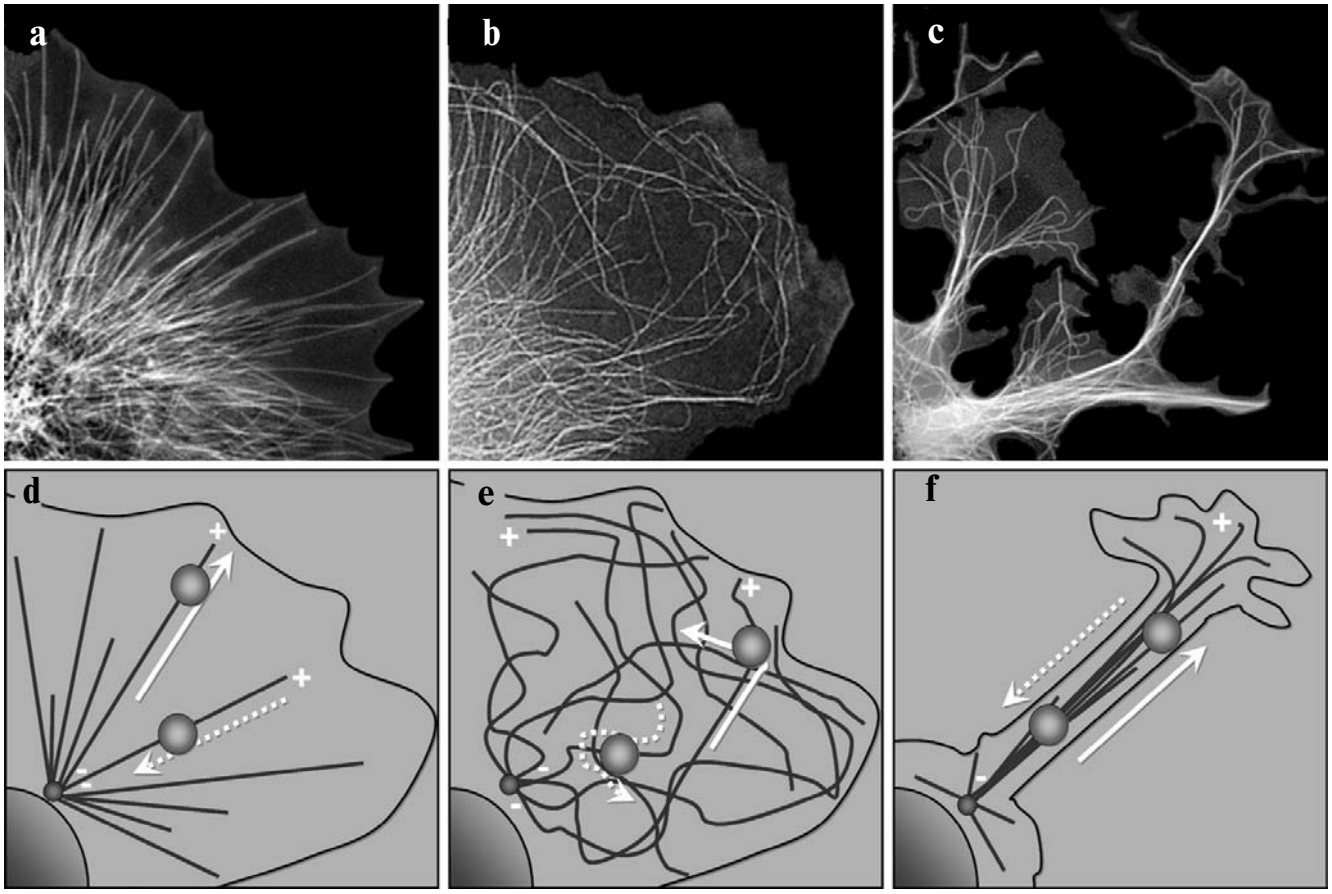
Cell components move in the cytoplasm due to two main reasons. First, this is cell compartmentalization: each process takes place in a certain region, for example, transcription proceeds in the nucleus, while translation happens in the cytoplasm, due to which RNA should be transported from the nucleus into the cytoplasm; synaptic vesicles have to be delivered from the neuron body to the axon end, and signalosomes formed in the synapse should be delivered back into the cell body. Second, it is the necessity for cell components to interact with each other, for which they have to come across each other in space. Naturally, all intracellular molecules and particles are subject to diffusion. However, particles and organelles (further called particles) have to overcome high cytoplasmic viscosity that interferes with their simple diffusion and may become an obstacle for transfer of big objects. Active transport is necessary to overcome significant distances in a reasonable time interval, and therefore it is especially important for large cells like neurons or oocytes.

Transfer of cell components along the cell is usually not similar, for example, to the purposeful movement of

ants along "ants' pathways". Most likely, outwardly it is the ant's chaotic movement along the ant-hill. Continuous purposeful flow of particles can be perhaps seen only in axons, but not in all cases. Usually a particle moves chaotically along the cytoplasm, often changing the direction of movement to the opposite several times a minute, and changes in direction alternate with periods of stationary state or "trembling", i.e. fine displacements around the same point. Only rarely there happen so-called saltatorial particle movements, brief "jumps" for several microns at the rate up to 2 μm/sec [5]. Measurements of particle movement rates also show that during short time intervals particles rarely develop high rates of transfer. Only 8-12% of mitochondria simultaneously (during 3-5 sec) move at a rate exceeding 0.2 μm/sec [7]. For example, the mean rate of transfer of mRNA-containing particles is ~0.1 μm/sec [8-12], i.e. it is 10 times slower than saltatorial movement.

Intensified mathematical analysis such as analysis of the particle mean squared displacement (MSD) dependence on the time interval [13-17] is necessary for exact characterization of movement of intracellular components. Particle movement is approximately described by the exponential equation $MSD(t) \sim Dt^{\alpha}$, where t is the time interval of observation, D is diffusion coefficient, and α is coefficient of anomalous diffusion. In the case of $\alpha < 1.0$ the particle movement can be considered as diffusion (with further subdivision to simple and obstructed diffusion), and in the case of $\alpha > 1.0$ it can be considered as active transport or directed movement [18]. It was supposed that a scheme when particles carry out low-amplitude transfers in some domains, rarely "jumping over" into adjacent domains, is the best suitable for intracellular particle transfers [17]. Probably in the case of microtubule-dependent movement, such domains can correspond to three-dimensional cells formed in the network of chaotically arranged microtubules (figure).

How can it be checked experimentally that we are dealing with active transport of some particles along microtubules? First, one should begin with clear identi-



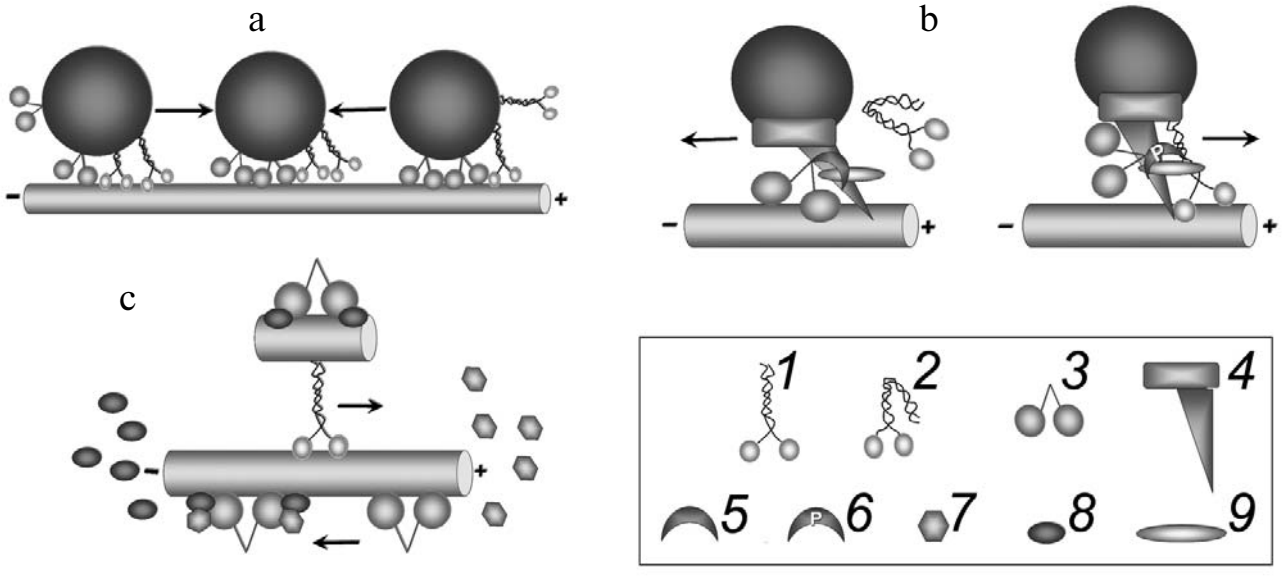
Microtubules and transport organization in different cell types. a-c) Microtubules in different cells. Fluorescent microscopy. d-f) Corresponding schemes of microtubule-dependent transport organization. a, d) Radial system of microtubules in *Xenopus* melanophore; b, e) chaotic system of microtubules in a cultured goldfish fibroblast; c, f) parallel bundles of microtubules in a *Xenopus* nervous system cell. The microtubule plus and minus ends are designated as well as possible trajectories of particles using kinesin (solid arrow) or dynein (dashed arrow) as motors

fication of the analyzed particles. At the present time there exists an opinion that, for example, membrane vesicles are represented in the cell by a great number of species differing in biochemical composition but morphologically similar. Second, it is necessary to make certain that tracks of the studied particle movement coincide with arrangement of microtubules and to find out that movement of particles ceases or is slowed down in the case of experimental destruction of the microtubule system (for example, by nocodazole). However, quite often studying the mobility begins with detection of direct interaction of any cellular protein with motor proteins. The special and most essential problem is identification of motor proteins involved in transport of different particles.

Active transport along microtubules involves motor proteins, mechanochemical ATPases belonging to two superfamilies—dyneins and kinesins [1-6, 19, 20]. These proteins interact via their ATPase domain with microtubules, while the opposite terminus interacts with transported cargo. Kinesins usually transport cargoes from the minus-ends of microtubules to their plus-ends, although minus-end-directed kinesins are also possible; dyneins transport cargoes from plus-ends of microtubules to minus-ends, i.e. in opposite direction. In the human genome, 39 kinesins are encoded, while kinesin-1 (KIF5 according to current nomenclature) and kinesin-2 (KIF3) are usually prevalent in interphase non-nerve cells [6]. Dyneins are not so diverse, but the cytoplasmic dynein molecule, in addition to heavy chains, includes intermediate, light intermediate, and various light chains, and some light chains can fulfill independent functions as individual proteins. Usually one and the same transported particle simultaneously carries several motor protein molecules of opposite charge, able to move both to plus and minus ends of the microtubule [2, 21, 22]. Sometimes particle movement resembles a "tug-of-war", when different motor molecules work simultaneously and try to tug the particles to different sides [22] (Scheme 1). It is believed that different motors can form complexes in common on transported loads.

There also exists passive transport along microtubules using ATP-independent one-dimensional protein diffusion. Such transport is shown for one of the kinesins—MCAK/XKCM1 (kinesin-13, KIF2C) [23]. Due to diffusion along a microtubule, MCAK quickly reaches its end, where it activates tubulin depolymerization in an ATP-dependent manner and thus increases the dynamics of the microtubule network [23]. The dynactin complex and possibly other proteins are also able to move along microtubules via one-dimensional diffusion (see below) [24], but this does not concern active transport, the subject of this article.

In cells with strictly determined arrangement of microtubules, the opposite sign motor proteins organize bidirectional transfer of organelles (figure) such as retrograde or anterograde transport in axons or dispersion and aggregation of pigment granules in melanophores of fish [25, 26]. In axons transport follows parallel microtubule oriented by their plus end in distal direction, and in melanophores along a radial network of microtubules where plus ends are directed to periphery and minus ends are assembled in the center (figure). However, in many other cell types microtubules are arranged quite chaoti-



Transport regulation in the presence of several motor proteins on the same particle. a) "Tug-of-war"; b) regulation using adapter protein phosphorylation (in this case of huntingtin); c) dynein transport by kinesin. a-c) Adapted from [22], [31], and [36], respectively. *1*) Active kinesin; *2*) inactive kinesin; *3*) dynein; *4*) dynactin; *5*) huntingtin; *6*) phospho-huntingtin; *7*) NUDEL; *8*) Lis1; *9*) HAP1.

Scheme 1

cally and particles can wander within the cytoplasm using only the single sign motor protein (figure).

Investigation of transport of different particle within cells includes studying the mechanism of particle complex formation with transporting proteins, selection of a certain protein, and binding to corresponding adapters. Various methods are used for identification of motor proteins involved in motility of any particle, e.g. intracellular motor and particle co-localization checked by immunofluorescence and synthesis of GFP-fused proteins. Interaction with the particle or its components is checked by co-precipitation in a yeast two-hybrid system, etc. Finally, knock-down techniques (gene switching-off using RNA interference), synthesis in cells of dominant-negative constructs, as well as injections of inhibiting antibodies or dominant negative-acting proteins are used.

Motility can be regulated via attachment and detachment of motor proteins as well as upon changing activity of these proteins, e.g. by reversible phosphorylation. Regulation by reversible phosphorylation can be used not against the motor protein molecule itself but against bound regulatory protein. Motor proteins actually include protein regulators, in particular, protein kinases and phosphatases [25], as well as regulatory structural proteins. Thus, the multisubunit dynactin complex is a regulator of dynein activity [27]. On one side, the dynactin complex associates dynein with the transported cargo, and on the other side, it provides for processivity of the motor at the expense of its $p150^{Glued}$ subunits [28, 29]. Protein $p150^{Glued}$ simultaneously interacts with tubulin of microtubules and with dynein intermediate chain, probably by helping the motor complex hold on the microtubule. Dynactin in frog melanophores binds both dynein and kinesin-2 and motors compete for binding to the same site in the $p150^{Glued}$ molecule [30].

Dynactin might be one of the key organizers of protein complexes on particles transported by motors. In neurons, the protein huntingtin is bound to dynactin, and huntingtin phosphorylation results in kinesin-1 recruitment into motor complex and dynein inactivation, which in turn, activates anterograde vesicular transport. On the contrary, huntingtin dephosphorylation results in kinesin release from the complex and activation of retrograde transport [31]. The complex also includes other proteins, in particular the structural protein Tau, one end of which interacts with microtubule and the other with dynactin [32].

The N-terminal domain of the DISC1 protein binds to kinesin-1, while the C-terminal domain of DISC1 binds to the NUDEL protein [33]. In this case, NUDEL interacts with dynein, dynactin, and the Lis1 protein [34-36]. Lis1 enhances dynein affinity to tubulin of microtubules, probably by dynein "sticking" to microtubule, while NUDEL is its antagonist (Scheme 1). This protein system is able to contribute significantly to the anterograde and retrograde transport regulation. It is found that proteins DISC1, huntingin, Lis1, and NUDEL are important for correct development and functioning of the nervous system.

The size of transported particle also plays a noticeable role in transport regulation because parameters of kinesin and dynein transport along microtubules (their mechanochemical cycle) depend on loading of these motors [37, 38].

mRNA TRANSPORT

After leaving the nucleus, mRNA and ribosomal subunits are transported within the cytoplasm until reaching their anchoring and translation sites, probably predetermined by some factors that will not be discussed in this review. A distortion in mRNA distribution in oocytes and somatic cells leads to impairment in cell functions of proteins synthesized with involvement of these mRNA. These distortions can be caused by microtubule depolymerization or upon inhibition of dynein or kinesin. This kind of data was obtained mainly on *Drosophila* oocytes and embryos. An important stage in detection of antero-posterior and dorso-ventral axes of its larva is nonrandom localization in oocytes of *Oskar*, *Bicoid*, and *Gurken* mRNA [39]. This nonrandom localization requires transport along microtubules.

Oskar mRNA and its translation product Oskar protein are located in the posterior part of the oocytes. In the case of microtubule destruction, they are evenly distributed within the oocyte cortex [40, 41]. Nonrandomness of *Oskar* distribution is also lost from *Drosophila* kinesin-1 null oocytes or containing mutant kinesin-1 with decreased activity. This confirms the role of the microtubule- and kinesin-1-dependent transport during mRNA distribution within oocytes. However, in oocytes there is no pronounced microtubule organization with their plus end direction towards the oocyte posterior part that could directly provide for *Oskar* transfer. Moreover, microtubules in oocytes are often directed by their plus-ends to the cell center, while their minus-ends are at the periphery, which would seem to make impossible transport by kinesin towards the cortex [41]. Initially, *Oskar* mRNA (like *Bicoid* and *Gurken*) is transcribed in feeder cells and then it is transported into oocytes through ring channels using dynein, binding to them via the BicD and Egalitarian proteins [42, 43]. Live cell imaging shows that *Oskar* mRNA in oocyte moves along microtubules [44]. In oocytes, *Oskar* mRNA associates with kinesin-1 by means of RNA-binding proteins HRP48, EJC, and Staufen, though this binding *in vivo* is not proved [40, 45-48]. The role of kinesin-1 in *Oskar* mRNA transport can be indirect, because kinesin-1 creates cytoplasmic flow in the oocytes that can transfer mRNA in the ooplasm, thus making easier its diffusion [44, 45, 47, 48]. Even low asymmetry of microtubular cytoskeleton in oocytes

(prevalence of microtubules in the oocyte posterior part) in the case of total and quite chaotic arrangement of microtubules and active mRNA transport along them can result in asymmetrical mRNA distribution and its accumulation in the oocyte posterior part [44]. It is possible that the role of kinesin-1 is *Oskar* mRNA transport from the periphery to the cell center at early stages of oocyte maturation, and this transport does not concern the oocyte posterior part in which significantly fewer microtubules depart from the cortex [41], i.e. kinesin-1 does not deliver mRNA to the site of its localization, but eliminates it from the sites of incorrect localization.

Kinesin-1 together with cytoplasmic dynein is necessary for antero–posterior localization of *Gurken* mRNA in oocytes [40, 43, 49]. *Gurken* mRNA is capable both of active transfer and anchoring on microtubules, using dynein, in sponge bodies of the anterior dorsal part of oocytes [49]. Quite often static *Gurken* transcripts are localized in the nucleus region on hyperstabilized nocodazole-resistant microtubules. Dynein or dynactin plays the role of a linker between microtubules and mRNA, because hyperexpression of dynamithin destroying the dynein–dynactin complex resulted in the loss of this interaction [46, 50]. Localization of *Bicoid* and *Wingless* mRNA and the Pair-Rule gene group (*Hairy*, *Runt*, and *Fushi tarazu*) transcripts in the anterior part of oocytes is also controlled by dynein [51, 52]. Localization of *Stardust* and *Crumbs* mRNA on the apical side of *Drosophila* follicular cells is controlled by dynein motor, and this process is important in epithelium polarization [53, 54]. Dynein can serve as a static "anchor" on microtubules for mRNA of *Runt* and *Fushi tarazu* genes in embryonal blastoderm [55].

Specific mRNAs are also actively transported in large cells of vertebrates. Thus, in *Xenopus* oocytes mRNA of Vg1 protein from the growth factor TGF-β family is transported by kinesin-2 [56]. This transport to the vegetative pole during oogenesis is necessary for the local expression of Vg1 protein, initiating processes of mesoderm and endoderm specification in frog embryogenesis [57]. Staufen protein, simultaneously binding to mRNA and kinesin, serves as adapter in this transport [58]. In mammalian neurons, kinesin-1 is involved in transport of mRNA of the calcium/calmodulin-dependent kinase CaMKIIα, Arc protein associated with actin filaments, and Tau protein associated with microtubules [59-61]. In oligodendrocytes, kinesin-1 also controls transport of mRNA of the main myelin protein MBP [62]. In the case of motor function distortion, mRNA localization is deteriorated in all cases, which usually results in distortion of functions of proteins translated from these mRNAs.

Direct observations of transport of individual mRNA molecules are complicated because it is difficult to obtain individual functionally active fluorescently labeled mRNA. To obtain such mRNA, *in vitro* RNA transcription using fluorescently labeled nucleotides is utilized. The resulting mRNAs are then introduced into cells by microinjection. This method has many limitations and has been used only in a few works. Granules formed by *in vitro* fluorescently labeled mRNA, introduced into cells by microinjections, demonstrate transference for significant distances at rates up to 1.0-1.5 μm/sec, which coincides with known values of rates that can be achieved by molecular motors. Such transfers depended mainly on cellular microtubule integrity [63]. To control the mRNA transference, there were also used mRNAs that form complexes with fluorescently labeled proteins having no intracellular functions of their own. To do this, a region containing 24 sites (19 nucleotides each) for binding phage capsid MS2 protein was introduced into the untranslated region (UTR) of the mRNA under study. This protein was synthesized in the same cells fused with GFP. The MS2 protein not bound to mRNA is sequestered in the nucleus and does not interfere with observations [64]. Live cell imaging of GFP–MS2–RNA complex confirmed that mRNPs are capable of active transfer within the cells, but along microtubules and only until the beginning of their translation [65, 66]. These experiments were carried out on cultured fibroblast-like cells, i.e. in such cells active mRNA transport along microtubules also takes place.

How are motor proteins bound to mRNA? Cytoplasmic dynein directly binds 3′-UTR in *Gurken* mRNA of *Drosophila* using its light chains [67]. For example, upon transport in neurons or in oligodendroglia, mRNA-containing particles reaching significant sizes are called transport granules. They include mRNA of various proteins as well as components of the protein-synthesizing apparatus. Proteins of ribonucleoprotein (RNP) particles can serve as mediators between motor proteins and mRNA. In rat hippocampus neurons, the GFP-labeled RNA-binding protein Staufen is transferred along the axon in a microtubule-dependent manner with frequent stops and direction inversion. As follows from results of immunolocalization and biochemical isolation, Staufen-containing particles are associated with both kinesin-1 and dynein. In addition to Staufen, they also contain different RNA-binding proteins [68]. In *Xenopus* oocytes, Staufen, due to binding to kinesin-1, coordinates *Vg1* mRNA transport to the vegetative pole [58]. Staufen functions were also necessary for positioning *Oskar* and *Bicoid* mRNA in oocytes and *Prospero* mRNA in *Drosophila* embryo neuroblasts [69-71]. In mammalian cells, Staufen is specifically associated with actively transported CaMKIIα, Arc, and MAP2 mRNA [72]. In mammalian neurons, β-actin mRNA is transferred along microtubules to the sites of active polymerization of actin [72], and Staufen knock-down lowers the β-actin expression level in soma and dendrites [73]. Staufen is probably able to bind β-actin mRNA and thus be involved in regulation of its transport.

In human cells, two Staufen orthologs as well as several splicing isoforms of these proteins are expressed. Their molecules contain several domains for double-stranded RNA binding, i.e. they can recognize elements of mRNA secondary structure. Besides, they contain domain for binding microtubules similar to the domain of microtubule-associated protein MAP1B [74, 75]. Probably binding to microtubules helps in transport of Staufen-associated mRNA along microtubules.

The molecular mechanism of development of a severe neurodegenerative disease, fragile X-chromosome syndrome (Martin–Bell syndrome), includes breaking the FMRP protein (fragile X mental retardation protein) mRNA transport along neuron dendrites. The FMRP-encoding mRNA is transported in the form of multicomponent complex that also includes FMRP proper. It appeared that inhibition of the dynein heavy chain or the kinesin-1 heavy chain inhibits transport of FMRP-containing particles to both sides in somatic *Drosophila* cells [76]. In this case, inhibition of kinesin-1 light chain has no consequences for the FMRP mRNA localization. However, in neurons the kinesin-1 light chains are necessary for transport of the FMRP-containing RNA granules [77]. Besides kinesin-1 and dynein, kinesin-2 (KIF3C) is also involved in transport of FMRP-containing particles [78]. Like Staufen, FMRP is able to bind to a particular set of mRNAs, and in this case FMRP recognizes G-quadruplexes. The FMRP-homologous protein FXRP1 modulates this interaction [79]. In addition to its own mRNA, FMRP interacts, for example, with mRNA of microtubule-associated protein MAP1B. Distortion of FMRP functions in neurons results in incorrect localization of this mRNA and increase in MAP1B expression, which in turn causes increase in microtubule stability and distortion of synapse functions [80, 81].

Another protein found within RNP particles is CPEB [82]. This protein specifically binds to and regulates translation of mRNAs that contain in their structure the elements of cytoplasmic polyadenylation (CPE). It appeared that the localized within the mitotic spindle translation of CPE-containing mRNA of mitotic kinesin Eg5, kinetochore protein CENP-E, and mitotic kinase Aurora is clearly controlled by CPEB and is necessary for chromosome segregation and meiosis in *Xenopus* oocytes [83]. In neurons, CPEB binds to CPE-containing mRNA and forms with them RNP particles transferred in a microtubule-dependent manner towards both plus and minus ends at the mean rate of 0.06-0.13 μm/sec [84]. According to results of immunolocalization and biochemical isolation, the CPEB protein-containing RNP particles are associated with both kinesin-1 and dynein. The kinesin-1 heavy chain and intermediate chain of cytoplasmic dynein are co-immunoprecipitated with CPEB, i.e. this protein can serve as adapter for both types of motors. Expression in neurons of CPEB mutant with deletion of a fragment necessary for interaction with the motors results in distortion of zonal distribution of CPE-containing mRNA of CaMKIIα and MAP2, but not of CPE-free mRNA of Arc, NF-M, and dendrin [84].

The Daz1 protein serves as an adapter between mRNAs and motors. The Daz1 protein gene is located in the Y chromosome locus associated with spermatogenesis pathologies determining male sterility [85, 86]. In cell cultures, GFP-labeled Daz1 forms particles transported along microtubules at mean rate 0.3 μm/sec. It appeared that activity of dynein–dynactin complex is required for this motility. Further biochemical experiments have shown that Daz1 is directly bound to the dynein light chains. Within the cellular mRNA pool Daz1 prefers transcripts whose untranslated regions are enriched with the $(GU)_n$-type repeats. Thus, it selectively binds to GU repeats in 5′-UTR of *Cdc25* mRNA and in 3′-UTR of *Mvh* mRNA. Overexpression in cells of Daz1 dominant negative mutant that lost the dynein binding site results in distortion of *Cdc25* and *Mvh* mRNA localization in spermatogonial mouse cells [87]. Thus, several sites for binding various motor proteins via different adapter proteins can be located on mRNP. This provides additional avenues for regulation of mRNP transport, especially for organization of their "wandering" within the cytoplasm in searching for the anchoring and translation sites.

TRANSPORT OF CYTOSKELETAL ELEMENTS

The cytoskeleton of eukaryotic cells consists of microfilaments, intermediate filaments, and microtubules. Unlike microfilament and microtubule proteins, those of intermediate filaments are insoluble under physiological conditions due to which there is a problem of ensuring the dynamics of intermediate filaments in dynamic cell cytoplasm. The intermediate filament protein vimentin forms short filaments, or structures looking like typographic tilde (squiggles) that are then incorporated into long vimentin fibrils. During fibroblast spreading, "tildes", not associated with any membrane structures, move bidirectionally along microtubules at the mean and maximal rates of 0.55 ± 0.24 and ~1 μm/sec, respectively [88, 89]. Intermediate filaments themselves are also able to move along microtubules, and kinesin-1 and dynein–dynactin complex are required for this [89, 90]. Motor proteins seem to extend the net of intermediate filaments along microtubule network. In this case, vimentin of intermediate filaments is able to interact directly with kinesin-1 and dynein–dynactin complex [88, 89, 91].

Neurofilaments or intermediate vimentin filaments are transported along axons in a similar way. The association of neurofilament protein peripherin with kinesin-1 and dynein was shown in experiments *in vitro* and *in vivo* [92]. Protein NUDEL1 is able to bind directly NF-L subunit of neurofilaments and regulate their assembly [93]. As mentioned above, NUDEL1 also binds dynein–dyn-

actin complex and protein Lis1. NUDEL1 knock-down in neuroblastoma cells inhibits retrograde transport of the intermediate filament precursors [94]. In regenerating axons, NUDEL1 (NDEL1) dissociates from NF-L and binds to a neuronal form of vimentin, which promotes regeneration [95].

Detailed video observations of slow axonal transport revealed microtubule-dependent motions of both intermediate filament precursors and short microtubule fragments themselves [96]. Microtubule fragments move with frequent stops and inversion of movement direction, which is indicative of the role of motors of both signs in the fragment transport. Short microtubule fragments can be kinesin cargo. In turn, cytoplasmic dynein, associated with such tubules, is their "passenger" for transporting in anterograde direction [97]. To retain dynein on the short microtubule fragment, protein Lis1 enhancing dynein affinity to tubulin of microtubules is used. Protein NUDEL1 that concentrates at the cell periphery is an antagonist of Lis1 and is induced release of dynein from the microtubules. Such dynein is loaded on the plus end of the microtubule along which it has quite recently moved as a "passenger" and now begins its typical movement in retrograde direction. Just such elegant mechanism was invented by nature and it seems that it is universal, because it occurs both in neurons and in cells of nonnervous origin [97]. In this case, tubulin of microtubules is delivered to the cell periphery where it is involved in dynamic assembly of microtubules.

In neuron cell culture, kinesin inhibition retarded anterograde transport of neurofilaments. In contrast, dynein inhibition retarded their retrograde transport as well as transfer from the cell body into the axon hillock [98]. Probably neurofilaments were associated with dynein that, in turn, was bound to short microtubules, as described above. Neurofilament phosphorylation contributed to their association with dynein, while dephosphorylation stimulated their binding to kinesin [98].

Another cytoskeleton component is the centrosome, the organizer of cellular microtubules. The centrosome consists of a pair of centrioles surrounded by pericentriolar matrix composed of fibrillar proteins. Pericentrin, ninein, and PCM1 are prevalent among the latter [99-101]. Proteins of the pericentriolar matrix provide for structural support of the main element that nucleates microtubules on centrosome, γ-tubulin ring complex γ-TuRC, also consisting of several proteins [102]. How do all these proteins accumulate in a small volume in the unique point of the cytoplasm? It appeared that pericentrin directly binds γ-TuRC, and then the complex is transported using dynein along microtubules to the centrosome. The direct dynein cargo in this system is pericentrin, binding to light intermediate chains of dynein [103]. A similar complex associated with dynein–dynactin and probably transported by the latter is formed by γ-TuRC with ninein [101, 104].

Another structural protein of pericentriolar matrix (PCM1) also forms particles that move in the microtubule-dependent manner towards the centrosome. The rates of PCM1-containing particle transfer (0.7-0.8 μm/sec) agree well with known rate values of dynein-dependent transport. Upon *in vitro* reconstruction of this system, PCM1-containing particles moved from the microtubule plus end to its minus end, and these motions stopped in response to specific dynein inhibitors [105]. As shown by the yeast two-hybrid screening, PCM1 binds to HAP1 protein (huntingtin-associated protein) that, in turn, directly interacts with the $p150^{Glued}$ subunit of the dynein–dynactin complex [106]. Recently discovered subcellular particles BBSomes, complexes of proteins, mutations in which result in development of Bardet–Biedl syndrome, bind to PCM1 [107, 108]. BBS proteins in complex with PCM1 are delivered to the centrosome where they dissociate from PCM1 and become involved together with protein Rab8 in formation of the primary cilium membrane [107]. Protein CEP290, associated with PCM1, is also involved in this process; mutations of CEP290 result in development of Jobert and Meckel–Gruber syndromes [109].

TRANSPORT OF STRUCTURAL PROTEINS

Compartmentalization of cellular space implies concentration of specific molecules in local cell regions. Active transport along microtubules is an ideal candidate for the role of a mechanism owing to which such concentration becomes possible.

The broad leading edge with pseudopodial activity and narrow retracting "tail" are distinguished in the body of a fibroblast moving along the substrate. Microtubules [110] and active kinesin-1 [111] are necessary to support this phenotype. It was supposed that directed delivery of some cellular components to the fibroblast active edge proceeds along microtubules with involvement of kinesin-1 [111]. Proteins regulating actin polymerization on the leading cell edge can serve as kinesin cargo, in addition to hypothetical vesicles incorporated in plasmalemma. Thus, it was shown that small GTPase Rac1 involved in regulation of lamellipodia formation on the cell active edge, as well as IQGAP1, a GTPase activator, also binding to actin, form complex with the kinesin-1 heavy chain. Uncoupling such complex results in distortion of typical localization of these proteins in the cellular cortex zone and, as a result, to anomalous behavior of the active edge of the cell [112].

Proteins WASP/WAVE, after binding to Arp2/3 protein complex, activate the latter and cause nucleation and arborization of actin filaments with formation of the "actin tree" type of structure, which results in formation of broad flat lamellipodia at the leading cell edge [113]. Disturbance of microtubule integrity or knock-down of

kinesin-1 results in the loss of typical WAVE2 distribution in the lamelloplasm zone. Results of immunolocalization and biochemical isolation show that WAVE2 forms a complex with kinesin-1 heavy chain together with protein kinase Pak1 and protein statmin/Op18, destroying microtubules [114].

Another example is kinesin-dependent transport of APC (*adenoma polyposis coli*) protein [115]. APC protein was first discovered as a tumor suppressor, mutations in gene of which play a decisive role in emergence of colon cancer. Congenital mutations of the *APC* gene also cause the inheritable oncological disease familial adenoma polyposis coli to be transformed into invasive cancer [116]. APC is directly associated *in vitro* and *in vivo* with cargo-binding subunit KAP3 of heterotrimeric kinesin (kinesin-2) [115]. Small APC-containing intracellular granules transported along microtubules can be observed. APC is also co-localized with the kinesin-1 heavy chains, and kinesin-1 depletion during RNA interference results the loss of peripheral localization of APC [117]. APC delivered to the cell periphery is able to interact with β-catenin, which results in degradation of the latter. β-Catenin can regulate epithelial–mesenchymal transition, i.e. morphological transformation of tumor cells by induction of mesenchymal marker expression or inhibition of epithelial marker expression [116]. Therefore, distortion of the APC-directed delivery to the sites of β-catenin accumulation can have serious consequences for the cell. APC is characterized by unusual variability of subcellular localization. It can be found near plasmalemma, in the nucleus, centrosome, mitochondria, and in mitotic spindle [118]. Some other cancer suppressors (BRCA1, BARD1) behave similarly, but they are not concentrated near plasmalemma and their direct interaction with motor proteins (at least up to now) has not been shown [118].

Catenin p120 also circulated between three functional cell domains—the cell nucleus, where it binds to the transcription repressor KAISO and thus causes derepression of responsive genes [119], intercellular contacts where, due to the posttranslational stabilization of E-cadherin it influences the strength of intercellular adhesion of epithelial cells, and the cell edge proper, at which it binds to the small GTPase Rho and, due to inhibition of its activity, influences the contractility of actomyosin cortex and cell motility [120]. It was found during video observations of GFP-p120 in living cells that GFP-p120 forms spots in the cytoplasm that are able to move with frequent stops and direction inversion. The following experiments have shown that this transport is provided for by kinesin-1. Catenin p120 directly binds to the kinesin-1 heavy chains *in vitro* and *in vivo*, and distortion of this interaction results in p120 accumulation in the nucleus and exhaustion of its pool in the region of intercellular contacts and free cell edge. The same consequences were produced by destruction of the microtubule system [121]. A similar phenotype is found in cancer of the pancreas [122].

Transport of structural proteins was shown for protein CG-NAP/AKAP350. CG-NAP is a multifunctional protein factor with double intracellular localization—it is localized on centrosome during mitosis and in Golgi complex in interphase. Its localization in Golgi complex appeared to be controlled by the microtubule system. Further investigations have shown that CG-NAP is transported to Golgi complex in the direction from the microtubule minus-ends using the dynein–dynactin complex. In cells, it interacts with $p150^{Glued}$ subunit of the dynein–dynactin complex, which is confirmed by data on immunolocalization and biochemical isolation. A CG-NAP mutant with deletion of the mapped $p150^{Glued}$-binding site cannot be transported to Golgi complex [123]. However, it cannot be excluded that catenin p120 and CG-NAP/AKAP350 are transported within the cell in association with small membrane vesicles, i.e. strictly speaking, their transport may be not related to transport of nonmembranous cell components.

TRANSPORT OF CELL SIGNALING PATHWAY COMPONENTS

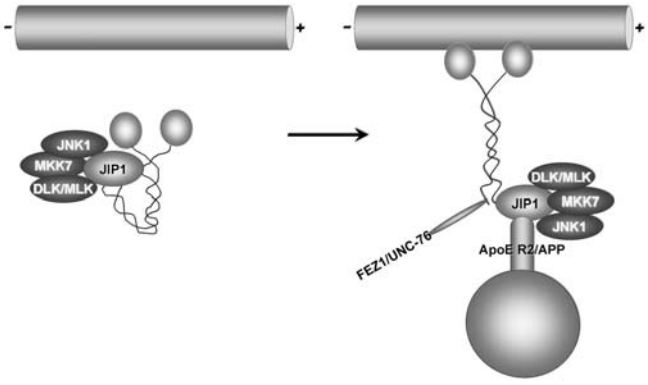
The intracellular system of microtubules includes both tubulin polymers and associated proteins influencing dynamic properties of microtubules. Besides, protein molecules, participants of the cell signal cascades (protein kinases, phosphatases, and transcription factors) are anchored at microtubules. Thus, the network of cellular microtubules can serve as a template for formation of clusters of signal molecules. In this case, cluster components can be associated with microtubules not directly, but via motor proteins or intermediate adapters.

Kinesin-1 binds to structural proteins JIP1-4 via its light chain TPR domain [124]. JIP1 forms a complex with JNK1, MKK7, and MLK kinases, members of the same signaling cascade. JIP3 similarly binds JNK3, MKK4/7, and MEKK1. In neurons, this provides for motility of the whole signal complex to the axon distal end. JNK1 activation causes destruction of JIP1 complex with kinesin, i.e. either inactive complex is transported, or there are mechanisms overcoming the action of JNK1. The kinesin-1 complex with JIP1 is also able to bind vesicular cargoes. It is possible that JNK1 activation takes place already in the synapse and results in release of both the JIP1–kinase complex and vesicles [125, 126]. Only JIP1 complex binding to kinesin-1 is not enough for activation of transport. Protein UNC-76/FEZ1 should bind to the kinesin heavy chains. This opens additional possibilities for regulation of the process [125, 127] (Scheme 2). JIP1 and JIP3 are transported in retrograde direction using the dynein–dynactin complex. JIP3 directly binds to protein $p150^{Glued}$ in dynactin. Besides, it organizes sig-

nal transduction from the points of nerve damage by transportation of activated JNK3 in retrograde direction [128].

Other members of the kinesin superfamily are also able to bind to signaling molecules. Pavarotti (KLP), the mitotic kinesin of *Drosophila*, necessary for formation of telophase spindle and contractile ring organization, binds polo protein kinase that plays an important role in centrosome functioning during cytokinesis. Pavarotti is responsible for delivery of polo to the centrosome and mitotic spindle during late anaphase [129]. Mammalian Pavarotti homolog, mitotic kinesin MKLP (KIF1B), also binds mitotic protein kinase Plk, a polo homolog [130]. The mixed-lineage kinase 2 (a member of MAP3K kinase superfamily) [131], PAR3 (incorporated in PAR3–PAR6–aPKC complex) [132], as well as protein phosphatase Dusp26 [133] bind, in particular, kinesin-2 (KIF3). The atypical protein kinase C complex (aPKC) with PAR6 and PAR3 proteins takes an important part in establishment of cell polarity, such as in fibroblasts and neurons moving along a substrate. The disruption of PAR3 interaction with KIF3 results in inhibition of neurite growth [132]. Protein phosphatase Dusp26 binds to motor KIF3A subunit of kinesin-2 and dephosphorylates its structural chain Kap3, which enhances β-catenin and cadherin binding to KIF3 and their delivery to the cellular contact sites [133].

Microtubules and motor proteins are used by transcription factors that run to the nucleus in response to signal transduction. The nuclear–cytoplasmic transport of transcription factors along microtubules is often regulated by kinases of microtubule localization. Thus, nuclear protein NF-κB is an inducible transcription factor activated in response to numerous external and internal stimuli. It is known that in the cytoplasm of most cell types NF-κB is constantly present within an inactive complex with IκBα. The inhibitory protein IκBα retains NF-κB in the cytoplasm due to masking of the sequence of its nuclear localization signal (NLS). The protein kinase C ζ-isoform and IKK kinase, associated with cytoplasmic microtubules, inactivate IκBα by its phosphorylation. In this case, IκBα itself is able to localize on microtubules and interact with dynein LC8 light chains [134-136]. This interaction protects IκBα against phosphorylation. In the case of intracellular redox-potential increase (for example, upon action of a cytokine from the family of tumor necrosis factors TNF-α), LC8 forms dimers with establishment of disulfide bonds and dissociates from IκB, thus allowing its phosphorylation and following degradation [135, 136]. Free NF-κB is imported into the nucleus and activates responsive genes. NF-κB itself is delivered to the nucleus along microtubules using the dynein–dynactin complex. At least in neurons the NF-κB p65 subunit directly binds via its NLS to the dynein intermediate chain, which is a critical condition for the NF-κB translocation into the nucleus in response to external signal [137]. It is interesting to note that in the case of TNF-α action, accompanied by NF-κB activation, hyperphosphorylation of the kinesin-1 light chains is also observed, which results in inhibition of activity of the latter. In this case, the cytoplasmic dynein remains active [138].



Kinesin-1 activation in complex with JIP proteins and other MAP-kinase cascade elements. Adapted from [125]. Designations as in Scheme 1

Scheme 2

Tumor suppressor p53 is also a quickly inducible transcription factor and in response to different signals, mainly concerning genomic DNA damage, it is capable of translocation into the nucleus. The exclusion of p53 from the nucleus and its accumulation in the cytoplasm are attributes of many tumor types. Owing to this, the p53 nuclear–cytoplasmic transport became the subject of intense attention of researchers. It was found that p53 is associated with cellular microtubules and is transported into the nucleus by cytoplasmic dynein [139]. It became clear upon mapping the p53–dynein binding site that the aberrant accumulation of p53 in the cytoplasm of many tumor cell types is associated with mutations in the C-terminal region of the protein. These mutations disturb the interaction of p53 with dynein [140].

Thus, the dynamics of nonmembranous cell components is a complexly organized and regulated process, the correct course of which is vital for a cell and the organism as a whole. From a technical point of view, observation of nonmembranous cell structures appeared even easier compared to membranous ones. Therefore, general regularities of active intracellular transport can be established on the example of membrane components.

It is necessary to consider microtubules and molecular motors as the most important link in the mechanisms of cellular signaling and regulation of gene expression. Transcription factors and protein kinases are main participants of the cell signaling cascades, and they exploit motors as the means of transport for their specific delivery or for action towards a localized substrate. Motor proteins on microtubules are able to separate one cascade from another to prevent crossing of signaling pathways.

This becomes possible due to the existence of specific intracellular adapter proteins forming unique multienzyme complexes. Therefore, often prevalent transport of any cellular component can be achieved upon hyperexpression of the latter without disturbance of transport of different cargos [125]. In these cases the limiting factor are adapter proteins without which the cargo loading on the motor protein is probably impossible. Motor proteins bind and transport a great number of various cell components. However, it is still not clear how the preference of some cargos to others is organized and whether the cargo choice is random or determined only by the law of mass action.

Active transport is involved in regulation of genome expression due to translocation of transcription factors into the nucleus, where they modulate gene activity, and due to mRNA transport within the cell and its localization in particular zones of the cytoplasm. It is clear that inhibition of various cellular motors inevitably causes pleiotropic effects, including those connected with disturbance in signal transduction, which should be considered in estimation of experimental results. Future trends of investigations should be connected with investigation of differential regulation of various intracellular transport pathways.

REFERENCES

1. Minin, A. A., and Kulik, A. V. (2004) *Uspekhi Biol. Khim.*, **44**, 225-262.
2. Welte, M. A. (2004) *Curr. Biol.*, **14**, R525-R537.
3. Gyoeva, F. K. (2005) *Mol. Biol. (Moscow)*, **39**, 709-718.
4. Caviston, J. P., and Holzbaur, E. L. (2006) *Trends Cell Biol.*, **16**, 530-537.
5. Moises, T., Dreier, A., Flohr, S., Esser, M., Brauers, E., Reiss, K., Merken, D., Weis, J., and Kruttgen, A. (2007) *Mol. Neurobiol.*, **35**, 151-159.
6. Hirokawa, N., and Noda, Y. (2008) *Physiol. Rev.*, **88**, 1089-1118.
7. Minin, A. A., Kulik, A. V., Gyoeva, F. K., Li, Y., Goshima, G., and Gelfand, V. I. (2006) *J. Cell Sci.*, **119**, 659-670.
8. Muslimov, I. A., Santi, E., Homel, P., Perini, S., Higgins, D., and Tiedge, H. (1997) *J. Neurosci.*, **17**, 4722-4733.
9. Rook, M. S., Lu, M., and Kosik, K. S. (2000) *J. Neurosci.*, **20**, 6385-6393.
10. Nielsen, F. C., Nielsen, J., Kristensen, M. A., Koch, G., and Christiansen, J. (2002) *J. Cell Sci.*, **115**, 2087-2097.
11. Scholz, D., McDermott, P., Garnovskaya, M., Gallien, T. N., Huettelmaier, S., DeRienzo, C., and Cooper, G., IV (2006) *Cardiovasc. Res.*, **71**, 506-516.
12. Scholz, D., Baicu, C. F., Tuxworth, W. J., Xu, L., Kasiganesan, H., Menick, D. R., and Cooper, G., IV (2008) *Am. J. Physiol. Heart Circ. Physiol.*, **294**, H1135-H1144.
13. Caspi, A., Granek, R., and Elbaum, M. (2000) *Phys. Rev. Lett.*, **85**, 5655-5658.
14. Caspi, A., Granek, R., and Elbaum, M. (2002) *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.*, **66**.
15. Weiss, M., Elsner, M., Kartberg, F., and Nilsson, T. (2004) *Biophys. J.*, **87**, 3518-3524.
16. Saxton, M. J. (2007) *Biophys. J.*, **92**, 1178-1191.
17. Destainville, N., Sauliere, A., and Salome, L. (2008) *Biophys. J.*, **95**, 3117-3119.
18. Bacher, C. P., Reichenzeller, M., Athale, C., Herrmann, H., and Eils, R. (2004) *BMC Cell Biol.*, **5**, 45.
19. Numata, N., Kon, T., Shima, T., Imamula, K., Mogami, T., Ohkura, R., Sutoh, K., and Sutoh, K. (2008) *Biochem. Soc. Trans.*, **36**, 131-135.
20. Amos, L. A. (2008) *Cell Mol. Life Sci.*, **65**, 509-515.
21. Pilling, A. D., Horiuchi, D., Lively, C. M., and Saxton, W. M. (2006) *Mol. Biol. Cell.*, **17**, 2057-2068.
22. Muller, M. J., Klumpp, S., and Lipowsky, R. (2008) *Proc. Natl. Acad. Sci. USA*, **105**, 4609-4614.
23. Helenius, J., Brouhard, G., Kalaidzidis, Y., Diez, S., and Howard, J. (2006) *Nature*, **441**, 115-119.
24. Culver-Hanlon, T. L., Lex, S. A., Stephens, A. D., Quintyne, N. J., and King, S. J. (2006) *Nat. Cell Biol.*, **8**, 264-270.
25. Kashina, A., and Rodionov, V. (2005) *Trends Cell Biol.*, **15**, 396-398.
26. Aspengren, S., Hedberg, D., Skold, H. N., and Wallin, M. (2009) *Int. Rev. Cell Mol. Biol.*, **272**, 245-302.
27. Schroer, T. A., and Sheetz, M. P. (1991) *J. Cell Biol.*, **115**, 1309-1318.
28. Waterman-Storer, C. M., Karki, S. B., Kuznetsov, S. A., Tabb, J. S., Weiss, D. G., Langford, G. M., and Holzbaur, E. L. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 12180-12185.
29. King, S. J., and Schroer, T. A. (2000) *Nat. Cell Biol.*, **2**, 20-24.
30. Deacon, S. W., Serpinskaya, A. S., Vaughan, P. S., Lopez Fanarraga, M., Vernos, I., Vaughan, K. T., and Gelfand, V. I. (2003) *J. Cell Biol.*, **160**, 297-301.
31. Colin, E., Zala, D., Liot, G., Rangone, H., Borrell-Pages, M., Li, X. J., Saudou, F., and Humbert, S. (2008) *EMBO J.*, **27**, 2124-2134.
32. Magnani, E., Fan, J., Gasparini, L., Golding, M., Williams, M., Schiavo, G., Goedert, M., Amos, L. A., and Spillantini, M. G. (2007) *EMBO J.*, **26**, 4546-4554.
33. Taya, S., Shinoda, T., Tsuboi, D., Asaki, J., Nagai, K., Hikita, T., Kuroda, S., Kuroda, K., Shimizu, M., Hirotsune, S., Iwamatsu, A., and Kaibuchi, K. (2007) *J. Neurosci.*, **27**, 15-26.
34. Feng, Y., Olson, E. C., Stukenberg, P. T., Flanagan, L. A., Kirschner, M. W., and Walsh, C. A. (2000) *Neuron*, **28**, 665-679.
35. Niethammer, M., Smith, D. S., Ayala, R., Peng, J., Ko, J., Lee, M. S., Morabito, M., and Tsai, L. H. (2000) *Neuron*, **28**, 697-711.
36. Sasaki, S., Shionoya, A., Ishida, M., Gambello, M. J., Yingling, J., Wynshaw-Boris, A., and Hirotsune, S. A. (2000) *Neuron*, **28**, 681-696.
37. Coppin, C. M., Pierce, D. W., Hsu, L., and Vale, R. D. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 8539-8544.
38. Brugues, J., and Casademunt, J. (2009) *Phys. Rev. Lett.*, **102**.
39. St Johnston, D. (2005) *Nat. Rev. Mol. Cell. Biol.*, **6**, 363-375.
40. Brendza, R. P., Serbus, L. R., Saxton, W. M., and Duffy, J. B. (2002) *Curr. Biol.*, **12**, 1541-1545.
41. Cha, B. J., Serbus, L. R., Koppetsch, B. S., and Theurkauf, W. E. (2002) *Nat. Cell Biol.*, **4**, 592-598.

42. Bullock, S. L., and Ish-Horowicz, D. (2001) *Nature*, **414**, 611-616.
43. Clark, A., Meignin, C., and Davis, I. (2007) *Development*, **134**, 1955-1965.
44. Zimyanin, V. L., Belaya, K., Pecreaux, J., Gilchrist, M. J., Clark, A., Davis, I., and St Johnston, D. (2008) *Cell*, **134**, 843-853.
45. Glotzer, J. B., Saffrich, R., Glotzer, M., and Ephrussi, A. (1997) *Curr. Biol.*, **7**, 326-337.
46. Januschke, J., Gervais, L., Dass, S., Kaltschmidt, J. A., Lopez-Schier, H., St Johnston, D., Brand, A. H., Roth, S., and Guichet, A. (2002) *Curr. Biol.*, **12**, 1971-1981.
47. Palacios, I. M., and St Johnston, D. (2002) *Development*, **129**, 5473-5485.
48. Serbus, L. R., Cha, B. J., Theurkauf, W. E., and Saxton, W. M. (2005) *Development*, **132**, 3743-3752.
49. Delanoue, R., Herpers, B., Soetaert, J., Davis, I., and Rabouille, C. (2007) *Dev. Cell*, **13**, 523-538.
50. Jaramillo, A. M., Weil, T. T., Goodhouse, J., Gavis, E. R., and Schupbach, T. (2008) *J. Cell Sci.*, **121**, 887-894.
51. Cha, B. J., Koppetsch, B. S., and Theurkauf, W. E. (2001) *Cell*, **106**, 35-46.
52. Wilkie, G. S., and Davis, I. (2001) *Cell*, **105**, 209-219.
53. Horne-Badovinac, S., and Bilder, D. (2008) *PLoS Genet.*, **4**, e8.
54. Li, Z., Wang, L., Hays, T. S., and Cai, Y. (2008) *J. Cell Biol.*, **180**, 31-38.
55. Delanoue, R., and Davis, I. (2005) *Cell*, **122**, 97-106.
56. Betley, J. N., Heinrich, B., Vernos, I., Sardet, C., Prodon, F., and Deshler, J. O. (2004) *Curr. Biol.*, **14**, 219-224.
57. Birsoy, B., Kofron, M., Schaible, K., Wylie, C., and Heasman, J. (2006) *Development*, **133**, 15-20.
58. Yoon, Y. J., and Mowry, K. L. (2004) *Development*, **131**, 3035-3045.
59. Aronov, S., Aranda, G., Behar, L., and Ginzburg, I. (2002) *J. Cell Sci.*, **115**, 3817-3827.
60. Kanai, Y., Dohmae, N., and Hirokawa, N. (2004) *Neuron*, **43**, 513-525.
61. Ohashi, S., Koike, K., Omori, A., Ichinose, S., Ohara, S., Kobayashi, S., Sato, T. A., and Anzai, K. (2002) *J. Biol. Chem.*, **277**, 37804-37810.
62. Carson, J. H., Worboys, K., Ainger, K., and Barbarese, E. (1997) *Cell Motil. Cytoskeleton*, **38**, 318-328.
63. Bullock, S. L., Nicol, A., Gross, S. P., and Zicha, D. (2006) *Curr. Biol.*, **16**, 1447-1452.
64. Querido, E., and Chartrand, P. (2008) *Meth. Cell Biol.*, **85**, 273-292.
65. Fusco, D., Accornero, N., Lavoie, B., Shenoy, S. M., Blanchard, J. M., Singer, R. H., and Bertrand, E. (2003) *Curr. Biol.*, **13**, 161-167.
66. Chang, L., Shav-Tal, Y., Trcek, T., Singer, R. H., and Goldman, R. D. (2006) *J. Cell Biol.*, **172**, 747-758.
67. Rom, I., Faicevici, A., Almog, O., and Neuman-Silberberg, F. S. (2007) *Biochim. Biophys. Acta*, **1773**, 1526-1533.
68. Villace, P., Marion, R. M., and Ortin, J. (2004) *Nucleic Acids Res.*, **32**, 2411-2420.
69. Roegiers, F., and Jan, Y. N. (2000) *Trends Cell Biol.*, **10**, 220-224.
70. Weil, T. T., Forrest, K. M., and Gavis, E. R. (2006) *Dev. Cell*, **11**, 251-262.
71. Broadus, J., Fuerstenberg, S., and Doe, C. Q. (1998) *Nature*, **391**, 792-795.
72. Bassell, G. J., Zhang, H., Byrd, A. L., Femino, A. M., Singer, R. H., Taneja, K. L., Lifshitz, L. M., Herman, I. M., and Kosik, K. S. (1998) *J. Neurosci.*, **18**, 251-265.
73. Goetze, B., Tuebing, F., Xie, Y., Dorostkar, M. M., Thomas, S., Pehl, U., Boehm, S., Macchi, P., and Kiebler, M. A. (2006) *J. Cell Biol.*, **172**, 221-231.
74. Wickham, L., Duchaine, T., Luo, M., Nabi, I. R., and DesGroseillers, L. (1999) *Mol. Cell. Biol.*, **19**, 2220-2230.
75. Furic, L., Maher-Laporte, M., and DesGroseillers, L. (2008) *RNA*, **14**, 324-335.
76. Ling, S. C., Fahrner, P. S., Greenough, W. T., and Gelfand, V. I. (2004) *Proc. Natl. Acad. Sci. USA*, **101**, 17428-17433.
77. Dictenberg, J. B., Swanger, S. A., Antar, L. N., Singer, R. H., and Bassell, G. J. (2008) *Dev. Cell*, **14**, 926-939.
78. Davidovic, L., Jaglin, X. H., Lepagnol-Bestel, A. M., Tremblay, S., Simonneau, M., Bardoni, B., and Khandjian, E. W. (2007) *Hum. Mol. Genet.*, **16**, 3047-3058.
79. Bechara, E., Davidovic, L., Melko, M., Bensaid, M., Tremblay, S., Grosgeorge, J., Khandjian, E. W., Lalli, E., and Bardoni, B. (2007) *Nucleic Acids Res.*, **35**, 299-306.
80. Lu, R., Wang, H., Liang, Z., Ku, L., O'Donnell, W. T., Li, W., Warren, S. T., and Feng, Y. (2004) *Proc. Natl. Acad. Sci. USA*, **101**, 15201-15206.
81. Menon, L., Mader, S. A., and Mihailescu, M. R. (2008) *RNA*, **14**, 1644-1655.
82. Richter, J. D. (2007) *Trends Biochem. Sci.*, **32**, 279-285.
83. Eliscovich, C., Peset, I., Vernos, I., and Mendez, R. (2008) *Nat. Cell Biol.*, **10**, 858-865.
84. Huang, Y. S., Carson, J. H., Barbarese, E., and Richter, J. D. (2003) *Genes Dev.*, **17**, 638-653.
85. Reynolds, N., and Cooke, H. J. (2005) *Reprod. Biomed. Online*, **10**, 72-80.
86. Brook, M., Smith, J. W., and Gray, N. K. (2009) *Reproduction*, **137**, 595-617.
87. Lee, K. H., Lee, S., Kim, B., Chang, S., Kim, S. W., Paick, J. S., and Rhee, K. (2006) *EMBO J.*, **25**, 4263-4270.
88. Prahlad, V., Yoon, M., Moir, R. D., Vale, R. D., and Goldman, R. D. (1998) *J. Cell Biol.*, **143**, 159-170.
89. Helfand, B. T., Mikami, A., Vallee, R. B., and Goldman, R. D. (2002) *J. Cell Biol.*, **157**, 795-806.
90. Gyoeva, F. K., and Gelfand, V. I. (1991) *Nature*, **353**, 445-448.
91. Liao, G., and Gundersen, G. G. (1998) *J. Biol. Chem.*, **273**, 9797-9803.
92. Helfand, B. T., Loomis, P., Yoon, M., and Goldman, R. D. (2003) *J. Cell Sci.*, **116**, 2345-2359.
93. Nguyen, M. D., Shu, T., Sanada, K., Lariviere, R. C., Tseng, H. C., Park, S. K., Julien, J. P., and Tsai, L. H. (2004) *Nat. Cell Biol.*, **6**, 595-608.
94. Shim, S. Y., Samuels, B. A., Wang, J., Neumayer, G., Belzil, C., Ayala, R., Shi, Y., Shi, Y., Tsai, L. H., and Nguyen, M. D. (2008) *J. Biol. Chem.*, **283**, 12232-12240.
95. Toth, C., Shim, S. Y., Wang, J., Jiang, Y., Neumayer, G., Belzil, C., Liu, W. Q., Martinez, J., Zochodne, D., and Nguyen, M. D. (2008) *PLoS One*, **3**, e2014.
96. Wang, L., and Brown, A. (2002) *Curr. Biol.*, **12**, 1496-1501.
97. Yamada, M., Toba, S., Yoshida, Y., Haratani, K., Mori, D., Yano, Y., Mimori-Kiyosue, Y., Nakamura, T., Itoh, K., Fushiki, S., Setou, M., Wynshaw-Boris, A., Torisawa, T., Toyoshima, Y. Y., and Hirotsune, S. (2008) *EMBO J.*, **27**, 2471-2483.

98. Motil, J., Chan, W. K., Dubey, M., Chaudhury, P., Pimenta, A., Chylinski, T. M., Ortiz, D. T., and Shea, T. B. (2006) *Cell Motil. Cytoskeleton*, **63**, 266-286.
99. Dictenberg, J. B., Zimmerman, W., Sparks, C. A., Young, A., Vidair, C., Zheng, Y., Carrington, W., Fay, F. S., and Doxsey, S. J. (1998) *J. Cell Biol.*, **141**, 163-174.
100. Dammermann, A., and Merdes, A. (2002) *J. Cell Biol.*, **159**, 255-266.
101. Delgehyr, N., Sillibourne, J., and Bornens, M. (2005) *J. Cell Sci.*, **118**, 1565-1575.
102. Raynaud-Messina, B., and Merdes, A. (2007) *Curr. Opin. Cell Biol.*, **19**, 24-30.
103. Young, A., Dictenberg, J. B., Purohit, A., Tuft, R., and Doxsey, S. (2000) *Mol. Biol. Cell*, **11**, 2047-2056.
104. Casenghi, M., Barr, F. A., and Nigg, E. A. (2005) *J. Cell Sci.*, **118**, 5101-5108.
105. Kubo, A., Sasaki, H., Yuba-Kubo, A., Tsukita, S., and Shiina, N. (1999) *J. Cell Biol.*, **147**, 969-980.
106. Engelende, S., Sharp, A. H., Colomer, V., Tokito, M. K., Lanahan, A., Worley, P., Holzbaur, E. L., and Ross, C. A. (1997) *Hum. Mol. Genet.*, **6**, 2205-2212.
107. Nachury, M. V., Loktev, A. V., Zhang, Q., Westlake, C. J., Peranen, J., Merdes, A., Slusarski, D. C., Scheller, R. H., Bazan, J. F., Sheffield, V. C., and Jackson, P. K. (2007) *Cell*, **129**, 1201-1213.
108. Loktev, A. V., Zhang, Q., Beck, J. S., Searby, C. C., Scheetz, T. E., Bazan, J. F., Slusarski, D. C., Sheffield, V. C., Jackson, P. K., and Nachury, M. V. (2008) *Dev. Cell*, **15**, 854-865.
109. Kim, J., Krishnaswami, S. R., and Gleeson, J. G. (2008) *Hum. Mol. Genet.*, **17**, 3796-3805.
110. Vasiliev, J. M., Gelfand, I. M., Domnina, L. V., Ivanova, O. Y., Komm, S. G., and Olshevskaja, L. V. (1970) *J. Embryol. Exp. Morphol.*, **24**, 625-640.
111. Rodionov, V. I., Gyoeva, F. K., Tanaka, E., Bershadsky, A. D., Vasiliev, J. M., and Gelfand, V. I. (1993) *J. Cell Biol.*, **123**, 1811-1820.
112. Takahashi, K., and Suzuki, K. (2008) *Exp. Cell Res.*, **314**, 2313-2322.
113. Chesarone, M. A., and Goode, B. L. (2009) *Curr. Opin. Cell Biol.*, **21**, 28-37.
114. Takahashi, K., and Suzuki, K. (2009) *Cell Signal.*, **21**, 695-703.
115. Jimbo, T., Kawasaki, Y., Koyama, R., Sato, R., Takada, S., Haraguchi, K., and Akiyama, T. (2002) *Nat. Cell Biol.*, **4**, 323-327.
116. McCartney, B. M., and Nathke, I. S. (2008) *Curr. Opin. Cell Biol.*, **20**, 186-193.
117. Cui, H., Dong, M., Sadhu, D. N., and Rosenberg, D. W. (2002) *Exp. Cell Res.*, **280**, 12-23.
118. Brocardo, M., and Henderson, B. R. (2008) *Trends Cell Biol.*, **18**, 587-596.
119. Daniel, J. M. (2007) *Biochim. Biophys. Acta*, **1773**, 59-68.
120. Xiao, K., Oas, R. G., Chiasson, C. M., and Kowalczyk, A. P. (2007) *Biochim. Biophys. Acta*, **1773**, 8-16.
121. Yanagisawa, M., Kaverina, I. N., Wang, A., Fujita, Y., Reynolds, A. B., and Anastasiadis, P. Z. (2004) *J. Biol. Chem.*, **279**, 9512-9521.
122. Mayerle, J., Friess, H., Buchler, M. W., Schnekenburger, J., Weiss, F. U., Zimmer, K. P., Domschke, W., and Lerch, M. M. (2003) *Gastroenterology*, **124**, 949-960.
123. Kim, H. S., Takahashi, M., Matsuo, K., and Ono, Y. (2007) *Genes Cells*, **12**, 421-434.
124. Verhey, K. J., and Rapoport, T. A. (2001) *Trends Biochem. Sci.*, **26**, 545-550.
125. Koushika, S. P. (2008) *Bioessays*, **30**, 10-14.
126. Horiuchi, D., Collins, C. A., Bhat, P., Barkus, R. V., Diantonio, A., and Saxton, W. M. (2007) *Curr. Biol.*, **17**, 1313-1317.
127. Blasius, T. L., Cai, D., Jih, G. T., Toret, C. P., and Verhey, K. J. (2007) *J. Cell Biol.*, **176**, 11-17.
128. Cavalli, V., Kujala, P., Klumperman, J., and Goldstein, L. S. (2005) *J. Cell Biol.*, **168**, 775-787.
129. Adams, R. R., Tavares, A. A., Salzberg, A., Bellen, H. J., and Glover, D. M. (1998) *Genes Dev.*, **12**, 1483-1494.
130. Liu, X., Zhou, T., Kuriyama, R., and Erikson, R. L. (2004) *J. Cell Sci.*, **117**, 3233-3246.
131. Nagata, K., Puls, A., Futter, C., Aspenstrom, P., Schaefer, E., Nakata, T., Hirokawa, N., and Hall, A. (1998) *EMBO J.*, **17**, 149-158.
132. Nishimura, T., Kato, K., Yamaguchi, T., Fukata, Y., Ohno, S., and Kaibuchi, K. (2004) *Nat. Cell Biol.*, **6**, 328-334.
133. Tanuma, N., Nomura, M., Ikeda, M., Kasugai, I., Tsubaki, Y., Takagaki, K., Kawamura, T., Yamashita, Y., Sato, I., Sato, M., Katakura, R., Kikuchi, K., and Shima, H. (2009) *Oncogene*, **28**, 752-761.
134. Crepieux, P., Kwon, H., Leclerc, N., Spencer, W., Richard, S., Lin, R., and Hiscott, J. (1997) *Mol. Cell Biol.*, **17**, 7375-7385.
135. Jung, Y., Kim, H., Min, S. H., Rhee, S. G., and Jeong, W. (2008) *J. Biol. Chem.*, **283**, 23863-23871.
136. King, S. M. (2008) *Sci. Signal.*, **1**, e51.
137. Mikenberg, I., Widera, D., Kaus, A., Kaltschmidt, B., and Kaltschmidt, C. (2007) *PLoS One*, **2**, e589.
138. De Vos, K., Severin, F., van Herreweghe, F., Vancompernolle, K., Goossens, V., Hyman, A., and Grooten, J. (2000) *J. Cell Biol.*, **149**, 1207-1214.
139. Giannakakou, P., Sackett, D. L., Ward, Y., Webster, K. R., Blagosklonny, M. V., and Fojo, T. (2000) *Nat. Cell Biol.*, **2**, 709-717.
140. Trostel, S. Y., Sackett, D. L., and Fojo, T. (2006) *Cell Cycle*, **5**, 2253-2259.