Journal of Cellular Biochemistry 101:1097–1108 (2007)

Cytoskeletal Elements and Intracellular Transport

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Abstract Recent advances in the understanding of the functions of various components of the cytoskeleton indicate that, besides serving a structural role, the cytoskeletal elements may regulate the transport of several proteins in the cell. Studies reveal that there are co-operative interactions between the actin and microtubule cytoskeletons including functional overlap in the transport influenced by different motor families. Multiple motors are probably involved in the control of the dynamics of many proteins and intriguing hints about how these motors are co-ordinated are appearing. It has been shown that some of the intermediate elements also participate in selected intracellular transport mechanisms. In view of the author's preoccupation with the steroid receptor systems, special attention has been given to the role of the cytoskeletal elements, particularly actin, in the intracellular transport of steroid receptors and receptor-related proteins. J. Cell. Biochem. 101: 1097–1108, 2007. © 2007 Wiley-Liss, Inc.

Key words: naER; HSP; dynein

The fundamental function of the cytoskeleton is to provide structural framework for the cytoplasm. Through such framework, the cytoskeleton is involved in various cellular organization and activities such as cell shape, distribution of cell organelle, cell motility, metabolism, and intracellular transport of macromolecules. The cytoskeleton is mainly composed of three distinct cytoplasmic fibrous structures namely microtubules (MT), actin filaments and intermediate filaments (IF). Along with these main structures, cells have a large number of motor proteins. Motors are molecular machines that move the cargo along actin and MTs. They include—(a) super family of myosins which move towards the plus (fast growing) end of actin filaments, (b) family of kinesins which are mostly positive end directed MT motors, and (c) family of dyneins which are negative end-directed MT motors. It was thought that MT and actin microfilaments have different cellular functions and constituents. Over the recent years it has become evident that the two are more interwined than was once thought to be [Lawrence et al., 1992]. In diverse cell types MT and actin filament networks cooperate functionally during a wide variety of processes like vesicle and organelle transport, cell migration and nuclear migration. Over the years, a growing number of cellular factors that bridge these cytoskeletal systems have been identified. These include hetero-motors complexes (physically associated myosin and kinesin), myosin-CLIP 170 complexes, forming homology proteins, dynein and the dynactin complex, Kar9p, coronin, Kelch repeat-containing proteins, and ERM proteins [Goode et al., 2000]. A relatively new family of unconventional myosin seems to grow and the functions attributed to them seem to expand. These actin-based motor proteins have been implicated in processes as seemingly diverse as endocytosis and transport of organelles. The regulation of intracellular membrane traffic is the common function of all [Tuxworth and Titus, 2000].

Abbreviations used: MT, microtubules; cyt D, cytochalasin D; EGF, epidermal growth factor; MHC, major histocompatibility complex; BCR, B cell antigen receptor; naER, non-activated estrogen receptor; HSP, heat shock protein; GFAP, glial fibrillary acid protein.

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Received 1 March 2007; Accepted 2 March 2007

DOI 10.1002/jcb.21347

To date 14 classes of unconventional myosins have been identified. Recent reports have implicated a number of these fibers in organelle transport and also in the formation, maintenance and dynamics of actin-rich structures involved in endocytosis, cell migration and sensory transduction [Wu et al., 2000].

ORGANELLE TRANSPORT

There are convincing examples of both actin and MT-based organelle transport in literature which raised the question of which pathway is used in a given situation. Earlier it was proposed that MT might be responsible for the long-range transport of organelle whereas actin might be responsible for local delivery [Atkinison et al., 1992]. Organelle transport is critical in neurons because the organelle may have to travel long distances along dendrites and especially along axons. Axonal transport is clearly dependent on MT, which are long and uniformly oriented with their positive end pointing away from the cell body [Heidmann et al., 1981]. Actin filaments, on the other hand, while abundant in axons, are considerably shorter, making them less suitable for axonal transport. It is being proposed that actin filaments participate in short range transport. For instance, they might bridge the gap between the MTs along the length of axon, tether organelles in specific location and/or be responsible for local transport in regions such as axon terminals where there are few MTs. A number of organelles undergo axonal transport and therefore there might be a variety of mechanisms involved (different motors or mixtures, different regulation, etc.). Myosin heavy chains have been grouped into about 15 classes. The classification is based on sequence similarity in the motor domains. Recently class V myosins have taken central stage co-operating with members of the kinesin family in neuronal transport, pigment transport in melanocytes and vacuole transport in yeast. This differs from other myosins in having a relatively high affinity for actin in the presence of ATP.

TRANSPORT IN NEURONS

Morris and Hollenbeck [1995] found that mitochondria used both MT and actin to travel in the neuritis of cultured neurons.

Tabb et al. [1998] isolated tubulovescicular elements from squid axoplasm that carried smooth endoplasmic reticulum (SER) marker. These elements showed affinity for both myosin V and kinesin in an in vitro assay system and moved along actin. In addition, actin-dependent (but not MT-dependent) motility of elements that SER in axoplasm was blocked by ant myosin V IgG. Genetic studies indicated that myosin V is also involved in actin-dependent organelle transport in dendrites. Several alleles of *dilute* (the mouse gene for myosin V) gave neurological defects in addition to the coat color defect. Purkinje cells in the cerebellum of these mice lack SER in their dendritic spines, although SER is present in the dendrites themselves. Since the spines lack MT and have a cortical network of actin, it is reasonable to propose that SER travels along MTs in the dendrites, and then moves to actin filaments for local transport into, or retention within, the spines. Allison et al. [1998] observed that actin filaments are essential for localization of glutamate receptors of the dendritic spines of cultured hippocampal neurons.

The transport of vesicles and the retention of organelles at specific locations are fundamental processes in cells. Actin filaments and myosin motors have been shown to be required for both of these tasks. Synaptic vesicles and their precursors also used both actin filaments and MT for various aspects of their transport. Precursors undergo axonal transport along MT employing several kinesin family members [Yonekawa et al., 1998]. Actin and myosin V may also be involved in the transport of precursors. Evans et al. [1998] isolated a population of vesicles from whole brain carrying both myosin V and synaptic vesicle protein. These vesicles could be activated to move along actin and an antibody against myosin V could block the movement. Myosins have been shown to have a major role in vesicle transport. Myosin V has been shown to transport endoplasmic reticulum vesicles in neurons, pigment granules in melanocytes and vacuoles in yeast. Myosin 1 is involved in the transport of Golgi-derived vesicles in epithelial cells. There is now strong evidence that two molecular motors, kinesin and myosin V, interact with each other and perhaps function as a complex on vesicles [DePina and Lang, 1999].

EXOCYTOSIS

Secretory granules are generated in the trans-Golgi network as immature secretory

granules, which undergo maturation process. These exhibit regulated exocytosis upon appropriate cellular stimulation. Living cell imaging revealed that newly formed immature secretory granules are transported in a direct and microtubule-dependent manner to the cell periphery [Rudolph et al., 2001]. This data also suggests that co-operativity of microtubules and actin filaments restrict these immature granules to the F-actin cortex where they move randomly and mature.

PHAGOCYTOSIS

The process of engulfing foreign particles (phagocytosis) is of fundamental importance for organisms from the simple unicellular organism that uses phagocytosis to obtain its next meal to complex metazoans in which phagocytic cells represent an essential branch of the immune system. All phagocytic processes are driven by a finely controlled rearrangement of actin cytoskeletons. It was shown that inhibition of actin polymerization by pharmacological agents prevented chemotactic motility, macropinocytosis, endocytosis and phagocytosis. The myosin family of motors interacts with the actin cortex to facilitate the internalization of external materials during early stages of phagocytosis. Recently, the members of kinesin and dynein motor families, which mediate transport along microtubules, have been shown to mediate the intracellular processing of the internalized materials. Some unconventional myosins are also involved in this process and it has been observed that the microtubule and actin-dependent transport systems might interact with each other [Ma et al., 2001]. The role of unconventional myosin in phagocytosis is recognized as a common denominator.

A study on phagosomal transport in mouse peritoneal macrophages suggests that there are two different transport systems of phagosomes in macrophages. Phagosomes smaller than 9 μm in diameter are probably transported to the perinuclear region by microtubules-based motility system and those longer than 3 μm by actin-based mechanism [Toyohara and Inaba, 1989]. Though the role of microfilaments, microtubules and intermediate filaments is unclear, it is evident that these units play an important role in macrophage function (migration, phagocytosis, and phagosome transport). Recent studies verify the roles of both actin filaments and microtubules in phagocytosis. When phagosome motion was studied by cell magnetometry using cytoskeletal drugs, the dominant factor for transport was shown to be the microfilaments [Moller et al., 2000]. Serrander et al. [2000] have recently investigated the role of gelsolin, a calcium-dependent actin severing and capping protein in blood neutrophils. Phagocytosis of IgG-opsonised yeast was reduced indicating that attachment and ingestion of IgG-opsonised yeast by murine neutrophils are actin-dependent.

Nitric oxide produced by inducible nitric oxide synthase (iNos) is an important component of host defense against intracellular pathogens. Sub-cellular localization of iNos with macrophages following phagocytosis revealed that they are seen adjacent to the peripheral cell membrane co-localized with the cortical actin cytoskeleton. Although not recruited to phagosomes, iNos association with the submembraneous actin is ideally suited to deliver NO to microbes in contact with the cell surface and may contribute to early killing of ingested *Salmonella* [Webb et al., 2001].

ENDOCYTOSIS

The endocytic compartment of eukaryotic cells is a complex intracellular structure involved in sorting, processing and degradation of a variety of internalized molecules. Recently the uptake through caveolae has been recognized as an alternative internalization pathway, which seems to be directly related to some signal transduction pathways [Pol et al., 2000]. A study in normal rat kidney (NRK) demonstrated that epidermal growth factor (EGF) causes partial redistribution of caveolin from the cell surface into a cellubrevin, an early endocytic compartment. Treatment of NRK cells with cytochalasin D (actin disrupter agent) or latrunculin A inhibited this pathway and the concomitant activation of mitosis activated protein kinase (MAP kinase). However if cells were pretreated with fillipin, cytochalasin D did not inhibit the phosphorylation of MAP kinase induced by EGF. The results concluded that in NRK cells the intact actin cytoskeleton is necessary for the EGF mediated transport of caveolin from the cell surface into the early endocytic compartment.

Quantitative electron microscopy and immunogold labeling revealed that though cytoskeletal elements are not of importance for endosome

maturation actin filaments facilitate fusion of mature endosome with preexisting lysosomes. However observations using electron microscopy and video microscopy of living cells showed that the concerned action of actin filaments and microtubules were responsible for the random distribution and movement of endocytic organelles throughout the cell. Actin microfilaments seem to facilitate perinuclear clustering and frequent fusion of mature endosome and lysosome. Microtubules play a role in preventing formation of large lysosome aggregation by separating endosome and lysosome and move them towards the cell periphery. Delivery of internalized molecules to lysosomes takes place by fusion of mature endosome with lysosome. Actin microfilaments have a major role in this step [VanDeurs et al., 1995].

Invasion of mammalian cells by the protozoan parasite, *Trypanosoma cruzi*, however, occurs by an actin-dependent mechanism distinct from phagocytosis. This parasite recruits host lysosomes to their attachment sites and lysosomal fusion serves as a source of membrane to form parasitophorous vacuole. Rodriguez et al. [1996] demonstrated that during this parasite invasion lysosomes are mobilized and its availability at the cell periphery requires microtubules and kinesin mediated transport. Myosin 1s, which is a ubiquitous monomeric subclass of myosins with actin based motor properties, is associated with plasma membrane and intracellular vesicles and has been proposed as a key player for membrane trafficking in endocytosis. Biochemical and immunoelectron microscopic evidence indicate that a pool of myosin 1-alpha, a member of the myosin 1s group is associated with endosomes and lysosomes. Overproduction of myosin 1-alpha or the production of non-functional truncated myosin alpha showed impairment of the delivery of fluid phase markers from endosome to lysosome [Raposo et al., 1999]. These authors suggested that myosin 1-alpha might be involved in membrane trafficking occurring between endosome and lysosome.

Newly synthesized MHC class II molecules associate with invariant chain (II) to form complexes. These complexes are transported to endosomes where proteolytic enzyme generates alpha/beta class II dimmers associated with nested II-derived peptides. These peptides are then exchanged with antigen peptides following which the mature class II molecules reach the cell surface. Role of actin in the transport and maturation of class II molecule has been studied [Barois et al., 1998]. It has been shown that cytochalasin D treatment of B cells caused a drastic reduction in the rate of degradation of B cells. This treatment also delayed appearance of stable form of class II molecules and reduced the presentation efficiency of antigen determinant requiring newly synthesized class II molecules. These authors suggest that dynamics of actin cytoskeleton can therefore control the meeting between newly synthesized class II molecule and lysosomal proteases involved in the degradation. Actin cytoskeleton is required for the trafficking of the B cell receptor (BCR) to the endosome [Brown and Song, 2001]. BCR plays central role in B cell activation. An early event in the B cell activation is the association of BCR with the actin cytoskeleton and an increase in F-actin has been shown to occur during this process. During B cell activation BCR helps to internalize signal transduction cascades that promote B cell to enter the cell cycle and facilitate antigen processing by accelerating antigen transport. Role of actin in BCR mediated antigen transport was studied using the actin filament disrupting agent, cytochalasin D (Cyto D) and an actin filament stabilizing agent, jsaplakinolide. Cyto D dramatically reduced the rate of internalization of BCR and blocked the movement of BCR from early endosomes to late endosomes/lysosomes without affecting BCR signaling. Thus BCR trafficking requires functional actin filaments for both internalization and movement to late endosome/lysosome.

Effect of Cyto D (an actin disrupting agent) and nocodazole (microtubule disrupting agent) on post endocytic traffic in Madin–Darby Canine kidney cells were analyzed [Maples et al., 1997]. Cyto D treatment inhibited basolateral to apical transcytosis of IgA in polymeric immunoglobulin receptor expressing cells. Like nocodazole, Cyto D acted at an early step in transcytosis and inhibited translocation of IgA between basolateral early endosome and the apical recycling endosome suggesting that in addition to microtubules post endocytic traffic in polarized epithelial cells also require actin filaments.

TRANSPORT OF BACTERIA AND VIRUSES

The host cell microfilaments and microtubules are known to play a critical role in the life cycle of several pathogenic intracellular

microbes. They are involved in the invasion and movement of pathogens once they are inside the host cell cytoplasm. *Orienta tsutsugamushi*, an obligate intracellular bacterium enters host cells by induced phagocytosis, escapes from the cytosol and replicates. It was shown that the cytosolic movement of the bacterium was mediated by microtubules [Kim et al., 2001]. By transfection studies it was shown that the movement was also mediated by dyneins, the microtubules related motor. Although the significance of this movement is not proven, the authors propose that the cytosolic bacteria use microtubules and dynein to propel themselves from the cell periphery to cytosol. Viruses spread in the host animal during pathogenesis, from their site of entry to distant site via the blood stream, lymphatic system and nervous system. As cytoplasmic diffusion operates only within very small volumes, active membrane traffic and cytosolic transport of viral genome-protein complexes are required. It is shown that such trafficking involves both actin and microtubules [Sodiek, 2000].

RNA TRANSPORT

There is now convincing evidence, largely from electron microscopy, immunohistochemistry and biochemical studies, which supports the concept, that mRNAs and polysomes are associated with the cytoskeleton [Hesekth and Prydme, 1991]. RNA cytoskeleton interactions influence transport, anchoring and translation of mRNA. Analysis of RNA movements in living cells suggests the formation of RNA granules and their active transport along microtubules [Bassell and Singer, 1997]. Recently a number of RNA binding proteins have been identified in flies, amphibians and mammals that are essential for the interaction of mRNA with actin and microtubules or actin–actin associated proteins. Such proteins include heterologous ribonucleoproteins which are also involved in the nuclear export of RNA [Jansen, 1999]. It has been suggested that in some instances it is a myosin motor, which translocates along actin microfilaments, and in others kinesin or dynein motors appear to be responsible for driving the movement of mRNA along microtubules [Stebbings, 2001].

PIGMENT GRANULE TRANSPORT

Fish and frog melanophores have long been studied as classical model system for microtubule based organelle transport. Upon stimulation pigment granules (melanosomes) disperse through the cell to cause the animal to become dark in color. A second stimulus causes the melanosomes to move into the cell center (aggregate) reversing the process. Microtubules radiate out from the cell center (centrosome) with a uniform polarity. These microtubules are required for both aggregation and dispersion of melanosomes. Later studies proved that actin was involved in these processes [Tuma and Getland, 1999]. Abolition of actin filaments did not inhibit aggregation or dispersion of melanosomes; however, it did not interfere with microtubules. For local movements melanosomes switch over to actin filaments.

The retinal pigment epithelial cells also exhibited regulated transport of pigment granules. Although the transport processes are similar to those in melanophores, here actin filaments (not microtubules) are required for aggregation and dispersion [Brown, 1999]. Like melanophores of lower vertebrates mammalian melanocytes also transport membrane-bound pigment granules (melanosomes). After the melanocytes arrive at the tip of the long melanocyte (dendrites) they are phagocytosed by neighboring keratinocytes. It was originally thought that the melanosome transport is solely actin based. Wu et al. [1998] have shown that melanosomes travel bi-directionally on microtubules. The actin filaments on the other hand serve to tether the melanosomes, concentrating them at the tip where they are phagocytosed. Involvement of myosin V in melanosome transport has also been shown [Wu et al., 2001].

Skin pigmentation is orchestrated through a series of complementary processes. One essential part is the translocation of melanosomes from perinuclear cytoplasm, towards the dendrite tips. Motor proteins use the energy derived from ATP hydrolysis for the movement and cooperative action of microtubules and actin filaments [Lambert et al., 1999].

STEROID RECEPTOR TRANSPORT

Steroid hormone receptors (SHRs) exact their effects after activation by their cognate ligands and binding to specific responsive elements on their target genes within the nucleus [Evans et al., 1998; Daniel et al., 2000]. Ligand binding regulates their association and dissociation by

altering the structure of the receptors and thus affecting the surface properties of the receptor [Renaud et al., 1995; Moras and Gronemeyer, 1998; Torchia et al., 1998; Marjaana et al., 2000]. The cellular localization of different SHRs is varied and dependent upon the specific receptor. Both the unliganded progesterone receptor and estrogen receptor are nuclear in localization, whereas unliganded glucocorticoid receptor (GR) exists within the cytoplasm and is translocated to the nucleus upon activation [King and Greene, 1984; Wikstorm et al., 1987; Perrot-Applanat et al., 1992; Daniel et al., 2000].

The glucocorticoid receptor exists in the cytoplasm of hormone-untreated cells as an oligomeric complex containing one molecule of receptor and two molecules of heat shock protein 90 (HSP90) [Pratt and Toft, 1997]. The receptor binds to actin filaments via the HSP90 moiety [Miyata and Yahara, 1991]. The authors suggest that the binding to actin may provide an anchoring mechanism for the receptor in the cytoplasm; whether it has any role in intracellular transport is not known. GR-hsp90 heterocomplexes isolated from cell lysates contain one of several hsp90-binding immunophilins that link the complex to cytoplasmic dynein [Galigniana et al., 2001; Davies et al., 2002], a molecular motor that processes along the microtubular tracks to the nucleus [Vallee and Gee, 1998]. The first evidence that the movement of a steroid receptor is dynein-dependent was shown by Jennifer et al. [2004]. This study has shown that over expression of dynamitin which is a component of the dynactin complex through which dynein links to vesicles and organelles, blocks movement by dissociating the dynein inhibiting the ligand dependent movement of the GR to the nucleus. Certain immunophilin binding proteins such as FKBP-51 and FKBP-52 have been shown to differentially regulate dynein interaction and nuclear translocation of the glucocorticoid receptor in mammalian cells [Todd et al., 2002; Gabriela et al., 2005].

Androgen, estrogen and progesterone receptors also form complexes with the heat shock protein (HSP90). All these receptor complexes might reasonably be expected to interact with actin filaments through their hsp90 moieties. Recent evidences have identified the actin-binding protein filamin, a 280 kDa component of the cytoskeleton, as a human androgen receptor (hAR) interacting protein. The functional significance of this interaction that was analyzed using filamin deficient cell line, via transient expression of a green fluorescent protein-hAR chimera, revealed that hAR remained cytoplasmic even after prolonged exposure to synthetic ligand [Ozanne et al., 2000].

A study on progesterone receptor showed that disruption of cytoskeletal networks did not prevent or delayed its nuclear translocation [Perrot-Applanat et al., 1992]. Karyophilic signals and interactions with the nuclear pore seem to be the primary determinants of the cellular traffic of the progesterone receptor.

Nirmala and Thampan [1992] purified a 55 kDa protein from the goat uterus, which transports estrogen receptor-alpha (ERα) to the nucleus. Its strong binding to tubulin-sepharose and actin-sepharose indicated the potential role of cytoskeletal elements in the nuclear transport. Non-activated estrogen receptor (naER), an alternative form of estrogen receptor with no DNA binding function, is localized on the plasma membrane of uterine cells and is internalized following estradiol binding [Karthikeyan and Thampan, 1996]. The binding to cytoskeletal elements facilitates the movement of naER towards the nuclear pore complex and this association may be influenced by the state of phosphorylation of the cytoskeletal structures [Zafar and Thampan, 1995]. This conclusion has been derived from the observations that in the presence of estradiol the phosphorylation of cytoskeletal proteins is drastically reduced. This is an observation that could possibly predict a limited interaction between the naER and the cytoskeleton since subsequent studies have indicated that interaction of naER with actin is mediated by a 58 kDa protein, p58 that mediates the nuclear transport of naER. Additional studies on this receptor have shown that clathrin coated vesicles containing naER is internalized following exposure of the membrane to estradiol [Sreeja and Thampan, 2004]. The internalized receptor is recognized by p58 that binds to the nuclear localization signals (NLS) on the naER on the one hand and actin filaments on the other. This binding appears to help in the vesicle movement along the cytoskeleton during the naER movement into the nucleus [Sreeja and Thampan, 2003a]. This study [Sreeja and Thampan, 2004] has confirmed that naER interaction with actin is clearly dependent on

the presence of p58. naER is a tyrosine kinase [Anuradha et al., 1994]. This activity is inhibited following naER exposure to estradiol. Zafar and Thampan [1995] observed that in rat uterus the cytoskeletal proteins actin and tubulin get phosphorylated in vitro, a response that is inhibited by exposure of the system to estradiol. This was an indirect indication for a possibility that the phosphorylation could have been influenced by naER since estradiol has a negative influence on its protein kinase functions.

Following nuclear entry naER dimerizes with a DNA binding protein, the estrogen receptor activation factor (E-RAF). The primary intracellular site where E-RAF is localized is the endoplasmic reticulum [Govind et al., 2003a,b]. E-RAF is an actin binding protein [Thampan et al., 2000]. It remains anchored to the endoplasmic reticulum through the mediation of two proteins. Anchor protein 55 (ap55) is a 55 kDa high affinity estrogen binding protein that is an integral protein of the endoplasmic reticulum membrane. Upon binding estradiol ap55 maintains a confirmation that promotes retention in its fold of a 66 kDa transport protein (tp66) which apparently recognizes the nuclear localization signals in the E-RAF and mediates in the E-RAF transport to the nucleus. This nuclear transport of E-RAF appears to be an actin directed movement since E-RAF is an actin binding protein. Exploiting this observation on the E-RAF binding to actin we recently designed a method by which E-RAF isolation could be achieved from goat uterine microsomes following exposure of the microsomes to 20 nM progesterone (Vidya and Thampan, unpublished observation). Progesterone binds to E-RAF, precipitating a conformational change in the protein. This apparently dissociates E-RAF from actin filaments facilitating E-RAF isolation through subsequent chromatography procedures. Some of the known functions of cytoskeletal proteins in steroid receptor movement are displayed in Table I.

Glutamate Receptor Targeting

Targeting of glutamate receptors (GluR) to synapses involves rapid movement of intracellular receptors. This occurs in the form of synaptic up regulation of receptors such as long term potentiation. Thus many GluRs are

TABLE I. Involvement of Cytoskeletal Elements in Intracellular Transport of Proteins

Proteins transported	Cytoskeletal elements	Motor system	Proteins involved	Area of movement	References
Glutamate receptor	Actin/MT	Myosin		Cytoplasm to post synapses	Petralia et al. [2001]
Neurofilament protein	MT	Kinesin		Cell body to axonal tip	Shea [2000]
Beta 1 integrin	Actin	Myosin	p13 kinase	Plasma membrane to axonal tips	Grabham and Godberg [1997]
Nerve growth factor	Actin/MT	Dynein	p13 kinase	Axonal transport	Reynolds et al. [1998]
Estrogen receptor (α)	Actin/tubulin		p55	Cytosol to nucleus	Nirmala and Thampan [1992]
Non-activated estrogen receptor	Actin		p58	Cytosol to nucleus	Sreeja and Thampan [2004]
Glucocorticoid receptor	Actin/MT	Dynein	HSP90	Cytosol to nucleus Cytosol to nucleus	Miyata and Yahara [1991]; Jennifer et al. [2004]
Androgen receptor	Actin	Filamin		Cytosol to nucleus	Daniel et al. [2000]
Glucose transporter	Actin		SNAP-23	Intracellular vesicles to plasma membrane	Dransfield et al. [2001]
IgG	Actin/MT		Gelsolin	Phagocytosis	Maples et al. [1997]
IgA	MT/actin			Interstitum to golgi	Crifo and Russo [1980]
MHC class II	Actin			Endosome to cell surface	Barois et al. [1998]
Beta cell antigen receptor	Actin			Plasma membrane to endosome	Brown and Song [2001]
Kar9p	Actin/MT	Dynein/myosin	BIMIP	Cytosol to nucleus	Beach et al. [2000]
Melanin	Actin/MT	Myosin		Cytoplasm to dendrite tips	Brown [1999]
Cathespin B	Actin/MT			Cellular compartment of chondrocytes	Zwicky and Baici [2000]
Phospholipase C gamma 1	Actin		EGF	Cytosol to plasma membrane	Wang et al. [2001]
Estrogen receptor activation factor	Actin	tp66 ap55		Endoplasmic reticulum to nucleus	Govind et al. [2003a,b]

retained in cytoplasmic pool in dendrites and are transported to synapses for up regulation. A recently held study shows that this process involves motor proteins such as myosin [Petralia et al., 2001].

Neurotrophin Transport

Neurotrophins are released from target tissues following neural innervations and bind to specific receptors situated on the nerve terminal plasma membrane. The neurotrophin-receptor complex undergoes retrograde axonal transport towards the cell soma, where it signals to the nucleus. There is good evidence for participation of actin cytoskeleton in neuroprotein axonal transport in vivo. Its also appears that the motor protein dynein mediates the transport of neurtrophin receptors which helps in targeting and trafficking of neurotrophins and facilitates the propagation of neurotrophin-induced-signals along the axon [Reynolds et al., 2000].

Role in Cholesterol Transport

Cholesterol is stored as ester in lipid droplets. Cholesterol ester is de-esterified and transported to mitochondria where steroid synthesis begins. Lipid droplets and mitochondria are attached to the intermediate filaments. Since these structures are not contractile, it appears to be necessary to invoke the action of other cytoskeletal elements. It is known that an energy dependent contractile process involving actin is capable of disrupting intermediate filaments. Intermediate filaments keep lipid droplets and mitochondria apart and disruption of filaments would be expected to allow the two structures to come together. This would open the way for transport of cholesterol to the steroidogenic pathway. Whether the event is necessary for the entry of cholesterol from the droplets into the mitochondria remains to be clarified. The calcium/calmodulin dependent protein kinase is shown to promote transport of cholesterol to mitochondria and does so under conditions in which phosphorylation of vimentin and myosin occurs. Phosphorylation alters the morphology and the functions of the cytoskeleton and this in turn is associated with accelerated cholesterol transport [Hall and Almahbobi, 1997]. Estrogen receptor activation factor (E-RAF), an actin binding protein, is a high affinity cholesterol and progesterone binding protein [Thampan et al., 2000]. Cholesterol or progesterone binding to E-RAF prevents the tp66 mediated nuclear entry of E-RAF since tp66, dissociates from E-RAF. Whether, under these conditions, E-RAF functions as an intracellular cholesterol or progesterone transport proteins remains to be examined. It also needs to be known whether E-RAF enters the mitochondria, in its function as a cholesterol/progesterone transport protein under these conditions. The presence of E-RAF in goat uterine mitochondria has recently been demonstrated (Vidya and Thampan, unpublished observation).

Other Transport Functions

Recent studies show that Na(+) H(+) exchanger requires an intact cytoskeleton for its optimal functions. Pharmacological interference with actin polymerization and myosin phosphorylation markedly inhibits the exchanger without altering the number of transporters exposed at the surface. The small GTP binding protein, Rho A and its downstream effector Rho kinase regulate the exchanger, possibly by controlling the level of myosin phosphorylation, that in turn regulates the organization of actin. Cytoskeleton is likely to sense physical alterations and transmit signal to modulate the exchanger activity, thus providing fast and effective control of the exchanger [Szaszi et al., 2000]. Cathespin B is a marketer for the differentiated chondrocyte phenotype and a potent mediator of cartilage metabolism in osteoarthritis. Both actin and microtubules are responsible for the transport of cathespin B between cellular compartments in chondrocytes [Zwicky and Baici, 2000].

Actin is involved in the propagation and regulation of insulin signals. In both muscle cells and adipose cells actin disassembly inhibited events such as recruitment of glucose transporter to the cell surface and enhanced glucose transport. Observations suggest that the actin cytoskeleton regulates proper subcellular distribution of signaling molecules that participate in the insulin signaling pathway [Tsakiridis et al., 1999].

Transport in Yeast

The actin cytoskeleton provides the structural basis for cell polarity in *Saccahromyces cerevisiae*. A polarized array of actin cables at the cell cortex is the primary structural determinant of polarity. Motors such as myosin V use

this array to transport secretory vesicles, mRNA, organelles, etc., toward growth sites where they are anchored by a cap of cytoskeletal and regulatory proteins [Pruyne and Bretscher, 2000]. Earlier studies show that many organelle transport events are actin-based and microtubules are required for mitosis and nuclear migration. All organelle transport in yeast serves one purpose-its growth by budding. Components, like organelles that are involved in cell wall synthesis, are shipped to predetermined locations at the cell surface to produce a bud. Recent studies reveal that these events employ myosin V as well as kinesin and dynein, which indicate the existence of co-ordination among the multiple motors systems.

Transport in Plants

The role of actomyosin complex has been clearly established for long-range mass transport in large algal cells and also in specialized cell of higher plants. This actomyosin complex act as a force generating system based on principles that operate as in muscle cells. Delicate meshwork built of short F-actin oligomers are critical for events occurring at the plasma membrane like exocytic and endocytic processes. Synergistic actions of actin-binding protein profiling and actin deploymerising factor ADF/cofilin are necessary for diverse aspects of plant morphogenesis. Rapid rearrangement of F-actin meshwork that interconnect endocellular membranes turned out to be especially important for signaling purpose in plant cells [Volkmann and Balsuka, 1999]. The plant Golgi apparatus plays a central role in the synthesis of cell wall material and also in the modification and sorting of proteins destined for cell surface vacuoles. Recent discoveries suggest that plant Golgi stacks can actively move through the cytoplasm along actin filaments [Nebenfuhr and Staehilin, 2001]. In recent years a few molecular motors have been isolated and characterized in plants. These studies indicate that some of the motors in plants have novel features and regulatory mechanisms. Analyses of the *Arabidopsis* genome sequence with conserved motor domains of kinesin and myosin families indicate the presence of a large number of molecular motors. The role of motors in cytoplasmic streaming, cell to cell communication, membrane trafficking and morphogenesis is beginning to be understood [Reddy, 2001].

Role in Hyphal Tip Growth

Hyphal tip growth is a complex process. It involves diverse intracellular movements, including interaction between the synthesis and expansion of cell wall and plasma membrane. F-actin is a major regulator and integrator of these processes. It directly contributes to

(a) the transport and exocytosis of vesicles that involve plasma membrane and cell wall materials,
(b) localization of plasma membrane proteins at the hyphal tips,
(c) cytoplasmic organelle migration and positioning.

The pattern of reorganization of F-actin prior to formation to new tips during branch initiation also indicates a critical role in early stages of assembly of the tip apparatus [Torralba and Heath, 2001].

Role of Intermediate Filaments

The main function of the intermediate filaments is to impact mechanical integrity to cells. However recent works show that they participate in some of the transport mechanisms. Intermediate filaments are subdivided on the basis of their amino acid and DNA sequence similarities. Type 1 and type 2 intermediate filaments are keratins. Type 3 includes vimentin, desmin, glial fibrillary acid protein (GFAP), peripherin and nuclear lamin.

Gillard et al. [1992] observed that globoside, a glycosphingolipid is co-localized with vimentin, desmin and keratin and GFAP. It was suggested that intermediate filaments participate in the intracellular transport and sorting of glycosphingolipids. Later studies by Gillard and colleagues proved that vimentin transported glycolipid and sphingolipid bases between the endosome/lysosome pathways and the Golgi apparatus and endoplasmic reticulum. Intermediate filaments accomplish this function by contributing to organization of sub-cellular organelles and/or by binding proteins that participate in the transport process [Gillard et al., 1998].

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