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- Review -

The Regulation Mechanisms of Kinesin Motor Proteins

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Proper intracellular transport is essential for normal cell function. Intracellular transport is mediated by microtubule-dependent molecular motor proteins, as well as kinesin and cytoplasmic dynein, which move their cargo along long, microtubule tracks in cells. Kinesins are ATP-dependent plus-end-directed motor proteins in the intracellular transport of organelles, vesicles, RNA complexes, and protein complexes. The mislocalization of these different types of cargo has been linked to cell dysfunction and degeneration. The cargo transport of kinesins can be described by the following steps: binding to the appropriate cargo and/or adaptor proteins, activation of the kinesin's motility and movement along the microtubule, and the release of the cargo at the correct destination. Recently, several studies have revealed the mechanisms for the regulation of kinesin motor activity, including cargo loading and unloading. Intracellular cargo transport is also modulated by adaptor proteins, which link the kinesins to their cargo. The regulatory proteins, which include protein kinases and phosphatases, regulate kinesin motor activity directly through the phosphorylation or dephosphorylation of kinesins and indirectly through the modification of adaptor proteins, such as c-Jun NH-terminal kinase-interacting proteins, or of the microtubule network. These findings lay the groundwork for understanding how kinesins are differentially engaged in intracellular cargo transport. In addition, understanding the regulatory mechanisms of each kinesin is an area of key interest within cell biology and neurophysiology. In this study, we reviewed kinesins' regulation proteins and discuss how their regulation affects cargo recognition and transport.

Key words: Adaptor protein, kinesin, phosphorylation, protein kinase, Rab protein

Introduction

The intracellular transport of membrane-bounded vesicles and organelles contributes for morphogenesis and functioning of the cell. Long-distance intracellular transport is dependents almost entirely on microtubule tracks [16]. Microtubules are polymerized with two subunits, α- and β-tubule at plus ends, organized in a radial array from the cell center toward the cell periphery [16]. In contrast to other cell types, microtubules are formed in a parallel unipolar array in the neuronal dendrites with plus ends oriented outward the cortex [17]. Two ATP-dependent microtubule motor proteins, kinesins and cytoplasmic dynein have directional motility on polar microtubule tracks in the cells [16]. Kinesins primary drive the transport of cargos along the microtubule tracks to pus-ends directly (Fig. 1). By contrast, cytoplasmic dynein associates an essential accessory proteins complex known as dynactin, which function transport of cargo to microtubule tracks to minus-ends directly [16]. Kinesins make up a large superfamily, with up to 45 members expressed in mammalian cells [34]. A standardized nomenclature groups kinesin genes into 14 subfamilies that share the motor domain similarity and the structural similarity [28].

The intracellular transport of various cargos in cells underlies many essential cellular functions, including the protein secretion, cell growth and cell signaling, trafficking of RNA complexes, protein and organelle degradation, and distribution of organelles. Kinesin-1 motors, a major motor for intracellular transport drive a wide range of cargos including various vesicles, mitochondria, and RNA particles [18]. Kinesin-2 motors drive the fodrin-positive vesicles [53], N-cadherin and β-catenin [54], and N-methyl-D-aspartate

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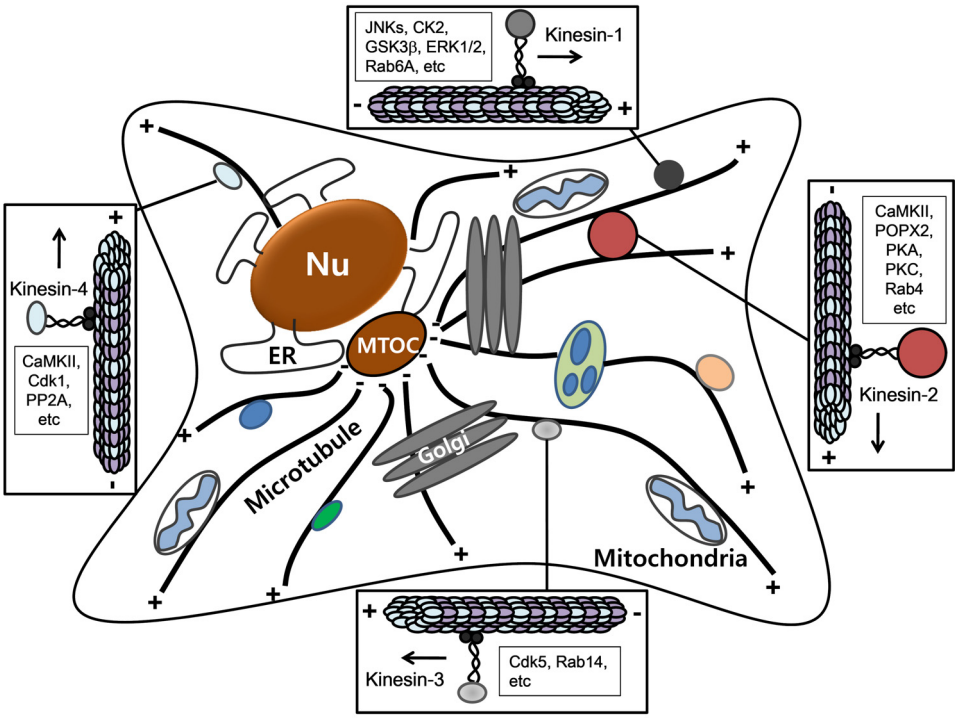

Fig. 1. Regulation of kinesins-mediated intracellular cargo transport by proteins kinases and Rab proteins. Kinesins drive the microtubule-dependent movement of organelles, vesicles, RNA granules, and proteins from the cell body to the cell periphery. Cargo-bound kinesins are regulated by several protein kinases and Rab proteins, such as glycogen synthase kinase 3 beta (GSK3β), c-Jun N-terminal kinase 3 (JNK3), extracellular signal-regulated kinases-1 and 2 (ERK1/2), protein phosphatase 2C-like serine/threonine phosphatases X2 (POPX2), cyclin-dependent kinase 5 (Cdk5), cyclin-dependent kinase 1 (Cdk1), Calcium/calmodulin-dependent protein kinase II (CaMKIIα), protein kinase A (PKA), protein kinase C (PKC), protein phosphatase PP2A, and casein kinase 2 (CK2). Plus (+) and minus (-) refer to the polarity of microtubules. ER; endoplasmic reticulum, Nu; nucleus, MTOC; microtubule-organizing center.

(NMDA) receptor containing vesicles in the cells [49]. Kinesin-3 motors drive the synaptic vesicle precursors and dense core vesicles [18]. Dysfunction of these kinesin-mediated cargo transports is relevant to abnormal neurogenesis and various neurological disorders such as Huntington's Disease (HD), Alzheimer's Disease (AD), Charcot–Marie–Tooth Type 2F (CMT2F), and Amyotrophic Lateral Sclerosis (ALS) [15].

The binding proteins of kinesins have been studied in detail to the specific cargo level. However, less is well studied about how the motor activities are regulated when bound to cargo and released it. The autoinhibition of kinesin-1 is an example of this regulation (Fig. 1) [4]. Kinesin-mediated cargo transport is also modulated by adaptor proteins. [18]. Adaptor proteins can effectively control the directionality of cargo-associated motor [50]. Kinesins and adaptor proteins directly bind to the modification proteins, including protein kinase, phosphatase, and small G-proteins (Table 1) [10]. Theses kinesin regulation mechanisms by autoinhibition or kinesin modification proteins allow for sustained the cell homeostasis [4].

In this review, we discuss the recent progress regarding the following questions. How do kinesins regulate loading and unloading their cargo? How do kinesins regulate the binding to microtubules? What is the biological significance of these regulation mechanisms?

Kinesin-1

Kinesin-1 is a tetramer consisting of two kinesin heavy chains (KHC; KIF5A, KIF5B, and KIF5C) that contain the motor domain and two kinesin light chains (KLCs) [23]. KLCs interact with the carboxyl (C)-terminal tail domain of KHC and regulate the cargo binding and the autoinihibition of the motor [4]. By binding with adaptor proteins or by direct interaction with cargos, kinesin-1 transport many different cargos, including mitochondria, mRNA-protein complex (mRNP), α-amino-3-hydroxy-5-methylisoxazole-4-proprionate

Table 1. Modification proteins interactions with Kinesins

Class	Kinesin family	Interaction protein	Adaptor protein	Cargo	Human Disease	References
Kinesin-1	KHC	JNK3, CK2, JNKs	JIPs	mRNP	Huntington	[6,26,47,51,61]
	KIF5A	Rab6A	BICDR-1	$GABA_AR$s	disease	[52]
	KIF5B	p38MAPK		AMPARs, mitochondria	SPG10	[62]
	KIF5C	CK2, p38MAPK, JNK3	JIPs		Alzheimer	[6,47,61,62]
	KLCs	LMTK2, ERK1/2, GSK3b, Rab27	Slp1		disease	[2,7,8,27,31,36,46,56]
Kinesin-2	KIF3A	CaMKIIa, PKA, POPX2		N-cadherin		[21,42]
	KIF3B	Rab4, Rab11	Rip11/FIP5	Intraflagellar transport (IFT)		[48,52]
	KIF17	CaMKIIa				
	KIF1A	DENN/MADD	Mint1	NMDARs		
				Synaptic vesicle precursors		[12] [38]
Kinesin-3	KIF1B-b	DENN/MADD	BICDR-1	MAGUK vesicles	Charcot-Marie-Tooth	[38]
	KIF1C	Rab6A	Radil		Disease type	[29]
	KIF13B	Cdk5	Radil		2A1	[60]
	KIF14	Rab14				[55]
	KIF16B	Rab14				[55]
Kinesin-4	KIF4A	CaMKII, Cdk1	PARP-1			[1,33]
	KIF7	PP2A				[30]
Kinesin-5	KIF11	Cdk5				[22]
Kinesin-8	KIF18	Cdk1, PP1g				[5,13]
Kinesin-13	KIF2A	PIPKa, TTBK2				[39,57]
	KIF2B	Plk1				[20]

(AMPA) receptor vesicles, γ-aminobutyric acid (GABA) receptor vesicles, and brain derived neurotrophic factor (BDNF) vesicles [18].

Phosphorylation

Kinesin-1 is phosphoproteins and the phosphorylation of KHCs can regulate the cargo loading and unloading [9]. Protein kinases can regulate the intracellular cargo transport through the direct phosphorylation of KIF5s or adapter proteins [11]. c-Jun N-terminal kinases (JNKs) are activated by cellular stress and regulate the intracellular transport by direct or indirect phosphorylation of kinesin-1, and adapter proteins [26]. JNKs directly phosphorylate KIF5B and induce its dissociation from microtubules [51]. JNKs also regulate the intracellular transport by phosphorylation of adaptor protein, such as JNK-interacting protein 1 (JIP1). In cells, phosphorylation of JIP1 at Ser 421 stabilizes its binding with kinesin-1 and activates the intracellular transport of amyloid precursor proteins (APP) containing vesicles [59]. These results show that JNKs-mediated phosphorylation of kinesin-1 and/or adaptor protein can modulate of the intracellular cargo transport in cells. Other JNKs, JNK3 blocks the intracellular transport by direct phosphorylation at Ser 176 of KIF5C, reducing microtubules binding affinity [6]. The motor domain of KIF5s contains the consensus JNK3 phosphotylation site corresponding to Ser 175 [6]. Phosphorylation of this residue has been implicated in HD [11]. Placement of negative charge at Ser 175 of KIF5s leads to a lower stall force and decreased velocity of motor. However, the ATPase activity, and microtubule-binding affinity are unchanged between wild-type motor domain and mutant construct. A sedimentation velocity experiment showed that a mutant favored the autoinhibited conformation [6]. This result suggests that cargo is transported by phosphorylated kinesin-1. Another protein kinase, casein kinase 2 (CK2) directly phosphorylates at Thr 338 which is located in the non-motor region of KIF5C [47]. In cultured cells, reducing CK2 expression decreased the lipid droplet transport, consistent

with a deceased number of active kinesin-I [61]. This data indicates that the protein kinase CK2 upregulates kinesin-based intracellular transport and the activity of cargo-bound kinesin-1.

p38 mitogen-activated protein kinases (p38 MAPKs) signaling pathway plays important roles in skeletal myogenesis and is activated in response to pro-inflammatory cytokines [43]. p38 MAPK directly phosphorylates Ser 175/Ser 176 of KIF5C and thereby inhibits the fast intracellular transport of membrane-bound organelle [62]. p38 MAPK also phosphorylates the tail domains of neurofilaments (NFs) and regulates their attachment between neurofilaments and motor proteins [35]. Two isoforms of KLC have been identified in mammals; the neuronal tissue-specific KLC1 and the ubiquitous KLC2 [45]. KLCs were phosphorylated by various protein kinases including extracellular signal-regulated kinases-1 and 2 (ERK1/2), glycogen synthase kinase-3 beta (GSK3β), and lemur tyrosine kinase-2 (LMTK2). ERK1/2 is playing a key role in neuronal differentiation and is activated in response to mitogens and growth factors [46]. ERK1/2 directly phosphorylates Ser 460 of KLC1 and prevents binding APP-labeled vesicles to kinesin-1, leading to a decrease the APP-contained vesicle transport in neuron [56]. However, ERK1/2-mediated phosphorylation of KLC1 has no effect on the kinesin-1-mediated intracellular transport of other cargoes, including collapsin response mediator protein 2 (CRMP2) [56]. GSK3β was identified as a regulator of glycogen metabolism in cells [8]. GSK3β directly phosphorylate KLC2 and adapter proteins [36]. In mammalian cells, GSK3β acts as a negative regulator of the intracellular transport [7]. In *Drosophila*, overexpression of GSK3β inhibits the intracellular transport of mitochondria and APP-containing vesicles by kinesin-1 [7]. In squid axoplasm, GSK3β releases the cargos from kinesin-1 without influencing the microtubule binding or ATPase activity of KHCs [36]. GSK3β has also been shown to regulate the intracellular cargo transport through phosphorylation of adapter proteins. Collapsin response mediator protein-2 (CRMP-2) acts as adaptor protein between kinesin-1 and cargoes, including such as TrkB-containing vesicles [24]. GSK3β-mediated phosphorylation of CRMP-2 inhibits the binding with KLC1 and CRMP-2 and blocks the intracellular transport of kinesin-1 cargoes [2]. LMTK2 plays important roles as a susceptibility gene for prostate cancer [58]. LMTK2 modulates phosphorylation of KLC2 by GSK3β. Using small interfering RNA, loss of LMTK2 was reduced the binding affinity of the kinesin-1 and Smad2 cargo [31]. Thus, phosphorylation of KLCs and adaptor proteins by various protein kinases modulates the cargo loading and unloading to kinesin-1.

Rab GTPase

Rab proteins are a family of monomeric guanine nucleotide (G)-binding proteins which have emerged as regulators of the intracellular transport in cells (Fig. 1) [52]. Interestingly, Rab27A and Rab27B associate with the tetratricopeptide repeat (TPR) of KLCs via adaptor protein, Slps (synaptotagmin-like proteins) [2]. Inhibition of the Rab27a/Slp3/kinesin-1 complex formation impairs lytic granules secretion to the immune synapse [27]. This data indicate that the binding with Rab27 protein and kinesin-1 or adaptor proteins modulates the intracellular cargo transport.

Kinesin-2

Four kinesin-2 subfamilies exist: the heterotrimeric complex composed of KIF3A, KIF3B, KIF3C motor subunits and KAP3, the non-motor subunit, which binds the cargo and the homodimeric complex of KIF17 motor subunit [28]. Kinesin-2 drives the various cargoes such as fodrin-positive vesicles [53], N-cadherin and β-catenin [54], NMDA receptor vesicles [49], and associate with Rab7-positive late endosome [3].

Phosphorylation

Using quantitative phosphoanalyses, protein kinase A (PKA) and Calcium/calmodulin-dependent protein kinase II (CaMKIIα) directly phosphorylates Ser 689, 694, and 698 of KIF3A [21]. In cultured cells, protein kinase inhibitors treatment and transfection of mutagenesis constructs of KIF3A revealed that cargo transport was enhanced by phosphorylation of the KIF3A [21]. Also, phosphorylation of KIF3 was upregulated cargo-loading activity, the association of KIF3 and cargos. Protein phosphatase 2C-like serine/threonine phosphatases X2 (POPX2), a serine-threonine phosphatase, interact with KAP3. POPX2 dephosphorylates Ser 690 of KIF3A and KIF3A-S690A mutant blocks the cargo transport [42]. Thus, POPX2 acts as a negative regulator of the cargo transport. These results suggest that phosphorylation and dephosphorylation pair of KIF3 regulate cargo-loading in the intracellular cargo transport process. Interestingly, CaMKIIα also binds to the tail region of KIF17 and phosphorylates at Ser 1029 of KIF17. Phosphorylation of KIF17 dissociates

Mint1, adaptor protein from the KIF17 and releases the cargos from KIF17 [12]. This result suggests that phosphorylation of KIF17 is an important mechanism for cargo-unloading. Phosphorylation of KIF3 upregulates the cargo-loading activity. However, the phosphorylation of KIF17 upregulates the cargo-unloading activity. These contrasting results may represent differences in the regulation mechanism of cargo loading and unloading between kinesin-2 motors.

Rab GTPase

Protein sorting, targeting and recycling of endocytosed membrane proteins are regulated by various Rab GTPases, such as Rab4, Rab8, and Rab11 [52]. Rip11/FIP5 was originally identified as a Rab11-binding protein and act as adaptor protein that recruit various proteins that regulate membrane transport [44]. The siRNA-based protein knockdown experiment showed Rip11/FIP5 regulates the sorting of internalized receptors to a recycling pathway. Interestingly, the tail domain of KIF3B directly binds to Rip11/FIP5 [48]. This interaction between Rip11/FIP5-Rab11 complex and KIF3 suggests that Rab GTPases are involved in the regulation of kinesin-2-mediated protein transport.

Kinesin-3

Kinesin-3 comprises five subfamilies, namely the KIF1, KIF13, KIF14, KIF16, and KIF28 [28]. This family is reported to be monomeric [40] or homodimeric structure [18]. Kinesin-3 drives the synaptic vesicle precursors, which contain synaptic vesicle proteins such as synaptophysin, synaptotagmin, and Rab3A [18, 28].

Phosphorylation

Cyclin-dependent kinase 5 (Cdk5) is regulates several signal processes in the cell function, including cell growth, and cell migration [25]. The inhibition of Cdk5 activity inhibited the intracellular transport of membrane-bound organelles and secretory vesicles [25]. Cdk5 phosphorylates Thr-506, a residue located in the forkhead-associated (FHA) domain of KIF13B [60]. The inhibition of Cdk5 activity modulated the association of KIF13B and transient receptor potential vanilloid 1 (TRPV1). The overexpression of Cdk5 in cells was promoted TRPV1 intracellular transport by activating the motor-cargo loading [60]. This result indicates that phosphorylation of KIF13 is an important mechanism for cargo-loading and upregulates intracellular cargo transport to cell surface.

Rab GTPase

Rab3A is required for the intracellular vesicle transport of in cells [52]. Differentially expressed in normal and neoplastic cells (DENN)/mitogen-activated protein kinase-activating death domain (MADD) binds with the stalk domain of KIF1A and KIF1β. Rab3A indirectly associates with KIF1A and KIF1β via DENN/MADD (Rab-GEP) and regulates the interaction between KIF1A/KIF1β and DENN/ MADD [38]. GTP-Rab3A transports presynatic precursor vesicles more effectively than GDP-Rab3A [38]. This result suggests that Rab3A regulates the KIF1A and KIF1β-mediated cargo transport. Another Rab protein, Rab6A directly binds the motor domain and the C-terminal region of KIF1C [29]. This association of KIF1C and Rab6A inhibits the interaction between KIF1C and microtubule track and slows intracellular cargo transport to the cell surface [29]. The association of Rab6A also directly regulates the motor activity of KIF1C. Rab14 directly associates with KIF16B and regulates the Golgi-to-endosome trafficking of the fibroblast growth factor receptor (FGER) containing vesicles during embryonic development [55]. These results revealed that Rab proteins regulate the microtubule affinity and cargo-loading of kinesin-3.

Kinesin-4

Kinesin-4 can be classified into five subfamilies: KIF4, KIF7, KIF21, KIF27, and NcKIF21 [28]. Kinesin-4 acts in the intracellular cargo transport, the microtubule dynamics, and the cell signaling [18]. For example, KIF4A regulates the stabilization of microtubules during cell division [33], and KIF7 regulates Hedgehog (Hh) signaling at cilia [14], and KIF21 regulates microtubule growth and participates in organizing microtubule arrays at the cell edge [37].

Phosphorylation

Genetic study indicates that KIF7 regulates Hh signaling in vertebrates. Hh signaling leads to ciliary accumulation and affects the localization of transcriptional regulators in cells [41]. The protein phosphatase 2A (PP2A) interacts with KIF7 and promotes the dephosphorylation of KIF7. The dephosphorylation of KIF7 promotes the trafficking of KIF7 and Gli proteins to the tips of cilia and for the transcriptional output of Hh signaling. [30]. KIF4 regulates programmed

cell death by interacting directly with poly ADP-ribose polymerase-1 (PARP-1), a nuclear enzyme that modifies various nuclear proteins with poly ADP-ribosylation to maintain cell homeostasis. [1]. When cells are stimulated by membrane depolarization via Ca^{2+} influx into nucleoplasm, CaMKII induces the dissociation of KIF4 from PARP-1, resulting in upregulation of PARP-1 activity. After dissociation from PARP-1, KIF4 enters into the cytoplasm from the nucleus [33].

Other Kinesins

Phosphorylation

Kinesin-5 is a homotetrameric protein and essential role in the mitotic spindle [28]. Cdk5 is the protein kinase responsible for phosphorylating kinesin-5 at Thr 926, which is important for kinesin-5 to associate with microtubules. Cdk5-mediated phosphorylation of Kinesin-5 also associates preferentially with microtubules rich in tyrosinated tubulin [22]. This result provides that Cdk5-mediated phosphorylation of kinesin-5 regulates the localization of kinesin-5 on dendritic microtubules, as they are known to be less detyrosinated than axonal microtubules.

KIF2 is a member of the kinesin-13 and regulates microtubule dynamics at growth cones [19]. Phosphatidylinositol 4-phosphate 5-kinase alpha (PIPKα) directly binds KIF2A [39]. The microtubule-depolymerizing activity of KIF2A was enhanced in the presence of PIPKα *in vitro* and *in vivo*. PIPKα also suppresses the elongation of axon branches in a KIF2A-dependent manner [39]. Tau-tubulin kinase 2 (TTBK2) directly phosphotylates Ser 135 of KIF2A and inactivates microtubule-depolymerizing activity by phosphorylation of KIF2A [57]. TTBK2 depletion reduces microtubule lifetime and impairs the cell migration. Overexpression of non-phosphorylatable type KIF2A also reduces microtubule lifetime and slowdowns the cell migration [57]. Using quantitative phosphoanalyses by mass spectrometry, KIF2B had identified the multiple phosphorylation sites. Polo-like kinase 1 (Plk1) directly phosphorylates KIF2B at Thr 125 and Ser 204, and that these two sites regulate KIF2B function [20]. Phosphorylation of Ser 204 of KIF2B is required for the kinetochore localization, and phosphorylation of Thr 125 of KIF2B is required for KIF2B activity in the kinetochore-microtubule attachments [20]. These results provide that various protein kinases promote the phosphorylation of KIF2, and regulate the microtubule dynamics.

KIF18A, a member of the kinesin-8, plays critical roles in various cellular processes, including cell motility, cell division, microtubule dynamics and the intracellular transport [32]. KIF18A directly interacts with protein phosphatase 1 (PP1) through a conserved RVxF motif [5]. PP1 induces metaphase plate thinning by dephosphorylating KIF18A. PP1 and Cdk1 antagonistically regulate KIF18A. Chromosome attachment induces Cdk1 inactivation and kinetochore recruitment of PP1 [13]. This result provides that chromosome movement is regulated by phosphorylation and dephosporylation of KIF18A.

Conclusion and outlook

In this review, we have discussed how various protein kinases and many Rab GTPases control the kinesin motor activity, cargos are loaded to their motors, and the cargos are unloaded upon reaching their destinations. Kinesins and adaptor proteins are major targets for the regulation of intracellular cargo transport. The proteins regulating kinesins and adaptor proteins have been rapidly identified in recent years. Given the important role, it is not surprising that alterations in protein kinase and Rab GTPase activities block the intracellular cargo transport and may lead to several nervous disorders. Thus, to disclose clearly the different regulatory mechanisms involved in the intracellular cargo transport is major challenge for future research.

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초록 : Kinesin 모터 단백질의 조절 기전

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세포내 수송 기구는 세포의 작용과 생존에 필수적이다. 이러한 세포내 수송은 긴 미세소관을 따라서 운반체를 운반하는 미세소관 의존 분자 모터 단백질인 kinesin과 cytoplasmic dynein에 의하여 이루어진다. Kinesin은 ATP 의존적으로 미세소관의 plus-end방향으로 이동하는 모터 단백질로 세포내 소기관, 분비소포, RNA 복합체, 단백질 복합체들을 수송한다. Kinesins에 의한 다양한 운반체의 수송의 이상은 세포의 기능 이상과 연관된다. Kinesins에 의한 운반체 수송의 기본 단계는: 운반체 혹은 adaptor 단백질과의 결합, kinesin 기능 활성화와 미세소관을 따라서 이동, 그리고 올바른 위치에서 운반체와의 분리 단계로 나뉘어 진다. 최근의 연구결과들에서 kinesin 모터 기능 활성화, 운반체와의 결합, 운반체와의 해리 기전이 확인되고 있으며 세포내 운반체 수송은 kinesin과 운반체를 연결하는 adaptor 단백질에 의하여서도 조절된다. 단백질 인산화 효소, 탈 인산화 효소를 포함하는 kinesin 모터 활성 조절 단백질들은 kinesin의 인산화 혹은 탈 인산화를 통하여 직접적으로 세포내 수송을 조절하거나, c-Jun NH-terminal kinase-interacting proteins (JIPs)와 같은 adaptor 단백질들과 미세소관의 간접적 수식을 통하여 세포내 수송을 조절하기도 한다. 이러한 연구결과들은 세포의 기능과 형태 유지에 관여하는 kinesin에 의한 다양한 세포내 수송 조절 기전을 이해하는데 기초적인 토대가 된다. 또한 각각의 kinesin에 대한 조절 기전을 밝히는 것은 세포생물학과 신경생리학을 이해하는데 중요하므로 본 종설에서는 kinesin에 의한 세포내 수송을 조절하는 단백질과 kinesin과 수송체와의 결합이 어떻게 조절되는지를 고찰하고자 한다.