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Intracellular transport and kinesin superfamily proteins, KIFs: strucutre, dynamics, and functions

Nobutaka Hirokawa

Department of Cell Biology and Anatomy, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

The intracellular transport is fundamental for cell morphogenesis, functioning and survival. To elucidate this mechanism we have identified and characterized kinesin superfamily proteins, KIFs, using molecular cell biology, molecular genetics, biophysics, X ray crystallography and cryo-EM. KIFs transport various membranous organelles such as mitochondria (KIF1Balpha/KIF5s), synaptic vesicle precursor (KIF1A/KIF1Bbeta), NMDA type (KIF17) and AMPA type (KIF5s) glutamate receptors and mRNAs with a large protein complex (KIF5s) in neurons and other cells along microtubules and play significant roles on cellular function and survival including learning and memory and relate to human diseases. KIF2A, a unique middle motor domain KIF, plays a significant role in brain wiring by depolymerizing microtubules in growth cones and controlling extension of axonal branches. KIF3 is fundamental for left-right determination of our body through formation of monocilia in the node which rotate and generate leftward flow of extra embryonic fluid, nodal flow, conveying vesicular parcels containing Sonic hedgehog and retinoic acid toward left and determines left-right asymmetry. KIF3 also suppresses tumorigenesis by transporting N cadherin - beta catenin complex from cytoplasm which works as a transcriptional factor with T cell factor and enhances cell proliferation as a signaling molecule. KIF4 controls activity dependent neuronal survival during development. In terms of the mechanism of motility we identified the simplest monomeric motor, KIF1A and revealed that KIF1A can move processively along microtubules as a monomer by a biased Brownian motion and elucidated how KIF 1A move on a microtubule using a single molecule biophysics, optical trapping nanometry, cryo EM, and X ray crystallography. Thus, KIFs play a number of significant roles not only on various cellular functions, but also on higher brain functions, brain wiring, activity dependent neuronal survival, tumorigenesis and fundamental developmental events such as left-right asymmetry.

Keywords**:** kinesin superfamily proteins; microtubules; transport; motility

Correspondence: Nobutaka Hirokawa
E-mail: hirokawa@m.u-tokyo.ac.jp

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NGF presents tensile properties in human skin dermis

Frederic Bonte[1], Celine Viennet[2], Marc Dumas[1], Philippe Humbert[2]

[1]Lvmh Recherche, Saint Jean de Braye, France; [2]Laboratoire Ingenierie ET Biologie Cutanee EA3183, Besancon, France

Cultured human epidermal keratinocytes and dermal skin fibroblasts, synthetised and released all neurotrophins and expressed also their high affinity tyrosine kinase transmembrane receptors of the trk family and low affinity receptor p75. In the skin these molecules act both like neurotrophic molecule for skin innervation, sensory neurons as well as growth and survival factors for keratinocytes under UVB stress. Nerve growth factor (NGF), is a polypeptide secreted by keratinocytes and exerts also paracrine functions on other skin cells. Nerve growth factor is the prototype for the neurotrophin family of polypeptides wich contains also brain-derived neurotrophic factor (BDNF) and neurotrophin-3 and -4. Recently we observed that UV regulates neurotrophin secretion and some neurotrophins were able to regulate collagen and metalloproteases (MMP2 and 9) synthesis in human dermal fibroblasts. The present work investigates new functions of these molecules ie. isometric tensile strengths developed by tightened dermal equivalents composed of normal human dermal fibroblasts and type I collagen. Real-time measurement of isometric forces was done using GlaSboxÒ with and without addition of these neurotrophic factors (10 – 100 ng/ml). After 7 h, NGF and to a lesser extent BDNF stimulated isometric strengths in a dose dependant manner to a maximum of 2929 mN (+30.2 %) and 2500 mN (+12.6 %) respectively. This effect was noted both during the exponential phase of the strengths development corresponding to vigorous matrix remodelling, than during the equilibrium phase when strengths reach their maximum. In the same time TGF-beta (2.5 ng/ml) known as a positive tension inductor, increased the strengths to 3177 mN (+55%) but no effect was observed with others. Using cultured normal human keratinocytes, NGF Elisa dosage and TGF-beta as positive control, it has been demonstrated that glycosphingolipids of natural origin can stimulate epidermal NGF secretion (+38% at 25 µg/ml; +55% at 50 µg/ml). Since keratinocytes and fibroblasts release NGF and BDNF, express their receptors and respond to those soluble factors by increasing their tensive properties, we conclude that NGF, called tensotrophin, has a essential role on the biomechanical properties of the dermis which could explain part of its role in wound healing. Furthermore, its epidermal secretion stimulation by a pharmacological agent can be an interesting way to

regulate its dermal tensile properties in firming and anti-aging skin care products.

***Keywords*:** NGF; fibroblasts; skin; epidermis; collagen

Correspondence: Frederic Bonte
E-mail: fredericbonte@research.lvmh-pc.com

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Fibroblast activation protein and dipeptidyl peptidase IV influence cell adhesion, migration and apoptosis

Xin M Wang, Denise M T Yu, Geoffrey W McCaughan, Mark D Gorrell

Molecular Hepatology Unit at Centenary Instute of Cancer Medicine and Cell Biology, Faculty of Medicine, University of Sydney and Royal Prince Alfred Hospital, Sydney, Australia

Cell adhesion, migration, proliferation and apoptosis are essential to many cellular and pathological processes. Fibroblast activation protein (FAP) and dipeptidyl peptidase IV (DPIV) are closely related type II proteins. DPIV is downregulated in tumor cells whereas FAP is a marker for various tumors, expressed only on activated fibroblasts in tumors and healing wounds. In addition, in chronic liver injury FAP is selectively expressed by activated hepatic stellate cells (HSC) and myofibroblasts in tissue remodeling regions. FAP has DP activity, collagen type I (CN-I) specific collagenase activity and Gly-Pro specific endopeptidase activity. DPIV is ubiquitously expressed and binds to fibronectin (FN). To investigate cell adhesion, apoptosis, proliferation, wound healing and cell migration in vitro, green fluorescent protein (GFP)-FAP and DPIV-GFP fusion proteins and a GFP control were expressed in the 293T human epithelial cell line. The human activated HSC cell line LX-2 was also studied. In epithelial cells, cells overexpressing FAP showed reduced adhesion to plastic coated with CN-I, FN or Matrigel.

***Keywords*:** cell adhesion; migration; apoptosis; proliferation; dipeptidyl peptidase

Correspondence: Xin M Wang
E-mail: maggiewang2810@gmail.com

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Primary cilia in fibrosis associated with two models of polycystic kidney disease

Cynthia G Jensen[1], Sue R McGlashan[1], Alastair C Johnstone[2], Cherie Stayner[3], Michael R Eccles[3], Tony Poole[4]

[1]Department of Anatomy with Radiology, University of Auckland, New Zealand; [2]Institute of Veterinary, Animal and Biomedical Science, Massey University, Palmerston North, New Zealand; [3]Department of Pathology, University of Otago, Dunedin, New Zealand; [4]Section of Orthopaedic Surgery, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand

The two characteristics of polycystic kidney disease (PKD) are the development of cysts and fibrosis. Fibrosis, a common cause of renal failure, results from excessive interstitial fibroblast proliferation and extracellular matrix production. Although previous studies of PKD have concentrated on primary cilia defects of cyst epithelia, we hypothesize that abnormalities in fibroblast primary cilia lead to the excessive fibrosis characteristic of the disease. Our study aimed to characterize interstitial fibrosis and fibroblast primary cilia expression in two separate models of PKD – an autosomal dominant PKD mouse (Pkd1del34/del34) and a recently-described autosomal recessive PKD in sheep (Johnstone *et al*., 2005, N Z Vet J). Kidneys from E18.5 Pkd1(del34/del34) and wild-type mice, and from newborn wild-type and mutant sheep were fixed and wax-embedded, and sections were stained using H&E or Masson's trichrome. The mean percentage areas of fibrosis and interstitial cell counts were used to indicate the extent of fibrosis. Primary cilia were labeled using an acetylated alpha-tubulin antibody. Moderate-to-severe fibrosis, with increased fibroblast numbers, surrounded the cysts in both the dominant and the recessive mutant kidneys. Primary cilia were present on tubular epithelia and interstitial cells in the wild-type kidneys of both mouse and sheep. In Pkd1 kidneys, primary cilia were associated with both unaffected epithelia and cyst epithelia; there was no difference in length between epithelial cilia of the cysts and those of wild-type tubules. However, only a small proportion of Pkd1 fibroblasts expressed primary cilia. In contrast, in the sheep, the majority of cyst epithelia did not express primary cilia and interstitial cells expressed only stunted cilia. This is the first study to describe the fibrosis in two models of cilia-related PKD. The presence of primary cilia in cyst epithelia confirms reports that the dominant Pkd1 mutation results in a functional ciliary defect. On the other hand, in the recessive mutant sheep the absence of primary cilia suggests a structural ciliary defect.

***Keywords*:** primary cilia; polycystic kidney disease; fibrosis; mutant; sheep

Correspondence: Cynthia G Jensen
E-mail: c.jensen@auckland.ac.nz

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The abnormal accumulation of α-internexin turns on the neuronal death in dt mice

Kuangwen Tseng, Kuoshyan Lu, Chungliang Chien

Department of Anatomy and Cell Biology, College of Medicine, National Taiwan University, Taipei

Dystonia musculorum (dt) is a mutant mouse with hereditary sensory neuropathy, and a defective bullous pemphigoid antigen 1 (BPAG1) gene is responsible for this mutation. In present study, we examined the distribution of neuronal intermediate filament proteins in the central and peripheral processes of the dorsal root ganglia (DRG) in adult dt mice by different approaches. We found that not only BPAG1 but also α-internexin was absent in the DRG neurons in adult dt mice. To study the relationship between the absence of α-internexin and the progressive neuronal loss in the DRG of dt mice, we further cultured DRG neurons from embryonic dt mutants. Immunocytochemical assay of cultured DRG neurons from dt embryos revealed that α-internexin was aggregated in the proximal region of axons and juxtanuclear region of the cytoplasma, yet the other intermediate filament proteins were widely distributed in all processes. The active caspase-3 activity was observed in the dt neuron with massive accumulation of α-internexin. From our observations, we suggest that (1) the interaction between BPAG1 and α-internexin may be one of the key factors involved in neuronal degeneration, and (2) abnormal accumulation of α-internexin may impair the axonal transport and subsequently turns on the cascade of neuronal apoptosis in dt mice.

Keywords**:** neurofilament; internexin; degeneration; dorsal root ganglia; dt mice

Correspondence: Chungliang Chien
E-mail:clc@ha.mc.ntu.edu.tw

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Simulated hypergravity and weightless using superconducting magnet affects several tumor cell lines proliferation and cytoskeleton

Peng Shang, Airong Qian, Dachun Yin, Wei Zhang, Jianping Cao, Pengfei Yang, Zhe Wang, Zongcheng Tian, Lifang Hu

Department of Cell Biology & Pharmacology Faculty of Life Science, North western Polytechnical University, Xi'an, China

The out space is a special environments of low gravity or weightless. Astronauts flying into the space or returning to the earth will endure hypergravity and weightless. Weightless conditions may results bone demineralization at a rate of about 1% per month, atrophy of their skeletal muscle. In order to test and examine gravity-sensing mechanisms and gravity-responses, a special designed superconducting magnet, which can produce large gradient magnetic field (LGMF) to provide three gravity levels (0g, 1g and 2g), was employed to simulate space gravity environment. The proliferation of several tumor cell lines in different gravity level, including human osteoblast cell MG-63, human breast cell MCF-7 and human T cell leukemia Jurkat-E6, was detected by using cell counting kit after being exposed to LGMF for 24h, 48h respectively. The effects of LGMF on MG-63 cytoskeleton also investigated by using confocal microscopy. The results show that the effects of LGMF on different cell line are different. The proliferative abilities of MCF-7 cells are increased while Jurkat-E6 cells' are decreased after being exposed to LGMF for 48h. LGMF also induced the rearrangement of actin microfilament and tubulin microtubule of MG-63. In conclusion, the superconducting magnet offers ground based opportunities to study how different body forces acting on specimens in space. Moreover, the sensitivities of different cell line to LGMF are different and the mechanism is being further studied.

Keywords**:** simulated hypergravity; simulated weightless; superconducting magnet; proliferation; cytoskeleton

Correspondence: Peng Shang
E-mail: shangpeng@nwpu.edu.cn

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Polymerization and distribution on actin cytoskeleton in pollen grains of cytoplasmic male sterile line in wheat

Yaqin Yao[1], Gaisheng Zhang[2], Bei Li[1], Na Niu[2], Yingli Zhang[3]

[1]College of Life Science, [2]College of Agronomy, [3]College of Resource and Environment, Northwest Sci-Tech University of Agrculture and Forestry, Yangling, China

Wheat (*Triticum aestivum* L.) is one of the most important crops in the world. The cytoplasmic male sterile line (CMS) of wheat, the vegetative growth is usually normal, and the pollen grains are abortive, is widely used in hybrid wheat breeding. The actin cytoskeleton plays an important role in the control of plant cell structure, development, motility, intercellular communication and cytoplasm streaming in pollen tubes. The recent evidence indicates that the actin cytoskeleton was related to the male sterility. In this paper, to reveal the effects of the actin cytoskeleton to CMS and the distribution of the actin cytoskeleton in the sterile and fertile pollens, K77(2)-CMS, T77(2)-CMS and their maintiner 77(2) was used as material, and optimal treatment methods were applied, namely de-fat, breakdown of the pollen wall and cell membrane, fixation and staining of cytoskeleton components so on, and after sealing observation was carried out on microscope. The network structural features of distribution of the actin cytoskeleton in pollen grains were clearly observed, it is showed that there are significant differences in the rate of polymerization and the distribution of the actin cytoskeleton in the abortion pollen grains, K77(2)-CMS and T77(2)-CMS lines, and the fertile pollen grains, their maintiner77(2). In the fertile pollen grains,

the actin monomer (G-actin) polymerizes into filamentous actin (F-actin), the F-actin dependent upon actin-binding proteins to link the intact actin network, which is well-distributed whole of pollen. In both abortion pollen grains of K77(2)-CMS and T77(2)-CMS lines, the actin network was disrupted, but K77(2)-CMS was shown to have a less disruption than that of T77(2)-CMS, The distribution of the actin network in K77(2)-CMS pollen grains was similar to that of the actin network in the fertile pollen grains, except that some regions actin network disrupted and disappeared. F-actin in T77(2)-CMS pollen grains is disrupted to show short F-actin, and not actin network, which is centralized in the middle part of the pollen grains, no distribution near outermargin of the pollen grains. The abortion pollen of T77(2)- CMS was observed at later mononucleate pollen stage, the morphology of the pollen grains varied greatly . Pollen abortion of K77(2)-CMS was observed during later binucleate stage and trinucleate stage, the morphology ofthe pollen grains varied a little,The investigations indicate that the rate of polymerization and distribution of the actin cytoskeleton is positively related to extent of the abortion of CMS pollen grains. In addition, the mechanism of male sterility about reaction of actin cytoskeleton to CMS was discussed in this paper.

Keywords**:** actin cytoskeleton; cytoplasmic male sterile (CMS); pollen; wheat

Correspondence: Yaqin Yao
E-mail: yaoyaqin@yahoo.com.cn

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Purification and characterization of recombinant Stn1p and Ten1p

Wei Qian, Mingliang Zhang, Jinqiu Zhou
Institute of Biochemistry and Cell Biology, Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Stn1p displays genetic and physical interaction with Cdc13p and Ten1p, essential proteins implicated in controlling telomere integrity. In order to address whether direct physical interaction exists among Cdc13p, Stn1p and Ten1p, a baculovirus system was used to co-overexpress these three proteins in insect cell. Co-pull down assay revealed that physical association was detected between Ten1p and Stn1p, or Ten1p and Cdc13p,but not detected between Stn1p and Cdc13p. Co-overexpressed recombinant Stn1p and Ten1p in insect cells were co-fractionated in a size column, indicating that Stn1p and Ten1p interacts directly. Their DNA binding activity and effect on telomerase activity are under investigation.

Keywords**:** Stn1p; Ten1p; Cdc13p; telomere

Correspondence: Wei Qian
E-mail: weiq@sibs.ac.cn

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The effect of nuclear M-CSF on the cell proliferation and the cell movement in Cos7 cells

Hongmei Luo, Xiaohong Zhang, Xueqin Zhao, Jian Tu, Yuhua Wang, Zewen Cui, Xiaoyong Lei, Shengsong Tang
Institute of Pharmacy and Pharmacology,Nanhua University, Hengyang 42100, China

Macrophage colony-stimulating factor (M-CSF) is an important growth factor that is involved in the genesis and progress of several diseases such as gynecologic malignancy, leukemia and hepatocellular carcinoma. M-CSF has soluble (sM-CSF), membrane-bound (mM-CSF) and cytosol and nuclear M-CSF (cnM-CSF) isoforms, Interestingly, cytosol and nuclear M-CSF was only identified pathologically in some leukemic cell lines and solid tumors as mammary carcinoma and HCC, the specific biological function of the nuclear M-CSF is not well understood. To help define the role of nuclear M-CSF, we constructed a eukaryotic expression plasmid pCMV/myc/nuc/M-CSF that target rnM-CSF to cell nuclei. After stably transfected into Cos 7 cell, Histomorphometric analyses indicated that a high expression M-CSF occurred in the nuclei of Cos 7 cells. The results show that the doubling time of pCMV/nuc/M-CSF-transfected cells, un-transfected Cos7 cells and pCMV/nuc/myc-transfected cells is 20.73±0.22 h, 28.22±0.25h and 27.88±0.24h, respectively, and that the growth rate of pCMV/nuc/M-CSF-transfected cells is greater than both pCMV/nuc/myc-transfected cells and un-transfected Cos7 cells. We also find that less and disorderly-arrangement microfilaments are occurred in pCMV/M-CSF-transfected Cos 7 cells, contrast to pCMV/nuc/myc- transfected or un-transfected Cos 7 cells, and nuclear M-CSF up-regulate cyclin B1, cyclinB2, cyclin A1, CDK4 and CDK2. Moreover, Cos 7 cells, which express nuclear M-CSF, have a stronger migration ability. We conclude that nuclear M-CSF stimulates the cell proliferation and movement of Cos 7 cells.

Keywords**:** Macrophage colony-stimulating factor; Cos 7 cell; cell movement; nuclear localization sequence; cytoskeleton

Correspondence: Shengsong Tang
E-mail: tangss111@yahoo.com.cn

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The effect of cytosol M-CSF on the cell proliferation and cell movement of NIN 3T3 cells

Hongmei Luo, Xiaohong Zhang, Leiyu Liu, Jian Tu, Yuhua Wang, Zewen Cui, Xiaoyong Lei, Shengsong Tang
Institute of Pharmacy and Pharmacology, Nanhua University, Hengyang 42100, China

Cytokines are multifunctional mediators that classically act by receptor-mediated pathways. Macrophage colony-stimulating factor (M-CSF) is a cytokine that is involved in the genesis and progress of several diseases such as gynecologic malignancy, leukemia and hepatocellular carcinoma. M-CSF has soluble (sM-CSF), membrane-bound (mM-CSF) and cytosol and nuclear M-CSF (cnM-CSF) isoforms, Interestingly, cytosol and nuclear M-CSF was only identified pathologically in some leukemic cell lines and solid tumors as mammary carcinoma and HCC, but detail functions of cytosol M-CSF in the processes of malignancy remain to be defined. To help define the role of cytosol M-CSF, we constructed a eukaryotic expression plasmid pCMV/cyto/myc/M-CSF that target mM-CSF to cell nuclei. After stably transfected into NIH 3T3 cell, Histomorphometric analyses indicated that a high expression M-CSF occurred in the cytosol of NIH 3T3 cells. The results show that the doubling time of pCMV/cyto/myc/M-CSF-transfected cells, un-transfected Cos7 cells and pCMV/cyto/myc-transfected cells is 26.23±0.32h, 37.71±0.25h and 39.63±0.24h, respectively, and that the growth rate of pCMV/cyto/myc/M-CSF-transfected cells is greater than both pCMV/cyto/myc-transfected cells and un-transfected Cos7 cells. We also find that less and disorderly-arrangement microfilaments are occurred in pCMV/cyto/myc/M-CSF-transfected NIH 3T3 cells cells, contrast to pCMV/nuc/myc- transfected or un-transfected NIH 3T3 cells cells. Moreover, NIH 3T3 cells, which express cytosol M-CSF, have a stronger migration ability. We conclude that cytosol M-CSF stimulates the proliferation and mobility of NIH 3T3 cells.

Keywords**:** macrophage colony-stimulating factor; NIH 3T3 cell; cell movement; cytoskeleton

Correspondence: Hongmei Wang
E-mail: tangss111@yahoo.com.cn

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Cellular responses induced by nuclear aggregates of non-polyglutamine protein

Lianwu Fu, Elizabeth Sztul
Department of Cell biology, University of Alabama at Birmingham, Alabama 35294, USA

Intracellular aggregation of misfolded proteins is associated with a number of human diseases. Cytoplasmic aggregates of proteins containing expanded polyglutamine (polyQ) tracks [exemplified by proteins responsible for neuro-degenerative diseases such as Huntington's disease (HD) and spinocerebellar ataxias (SCAs)], or proteins lacking polyQ stretches (exemplified by the amyloid-generating proteins responsible for Parkinson's disease and Alexander's disease) have been described. In contrast, nuclear aggregates are formed by polyQ proteins, suggesting that polyQ tracks might be relevant for the nuclear aggregation and the neuron pathogenesis in HD and SCAs. To explore the selectivity of nuclear aggregation, we examined whether a non-polyQ protein can form nuclear deposits. We investigated the effects of over-expressing a protein chimera consisting of the Green Fluorescent Protein (GFP) and a fragment of the Golgi matrix protein GCP170 (GFP170*) in cells. GFP170* accumulates in cytoplasmic aggresomes, and also deposits in discrete foci within the nucleus. Both, cytoplasmic and nuclear aggregates recruit a subset of cellular chaperones (Hsp70 and Hdj2), and the 20S proteasome. Formation of cytoplasmic and nuclear GFP170* aggregates requires an intact microtubule (MT) cytoskeleton. The nuclear deposition of GFP170* shows close spatial relationship with a nuclear matrix-associated structures containing the promyeolocytic leukemia (PML) protein. Deposition is initiated as numerous small foci in or adjacent to PML bodies. Time-lapse imaging shows extensive movements and fusion of small foci to form larger structures within the nucleus. The enlargement of the foci is paralleled by spatial rearrangements of PML bodies. These results indicate that nuclear deposition and nuclear rearrangements are not unique to polyQ containing proteins, and may represent a general response to foreign proteins gaining access to the nucleus. The nuclear inclusions of GFP170* recruit transcription factors such as CBP (CREB-binding protein) and p53. The aggregation of GFP170* represses p53 activity and induces cell death. Our results suggest that the cellular toxicity induced by nuclear aggregates are caused by the sequestration of certain transcriptional factors and are common responses to the nuclear aggregation of either polyQ or non-polyQ protein. We propose that some polyQ-independent mechanisms might contribute to the neuropathology in certain neuron degenerative diseases such as HD and SCAs.

Keywords**:** protein aggregates; protein aggresomes; polyglutamine; neuron degeneration; cell death

Correspondence: Lianwu Fu
E-mail: lianwufu@uab.edu

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CPAP/CENPJ: a novel microtubule depolymerizer that regulates microtubule dynamic instability in cells

Wen-Bin Hsu, Liang-Yi Hung, Chieh-Ju C Tang,

Tang K Tang
Institute of Biomedical sciences, Academia sinica, Taipei

Accurate chromosome segregation in mitosis is crucial for cell viability and normal function. The segregation of chromosomes requires the proper function of centrosome and spindle assembly. Defects in these processes can lead to aneuploidy and genomic instability. We previously reported a novel centrosomal protein CPAP (centrosomal P4.1-associated protein), which is associated with the gamma-tubulin complex. Recent studies of congenital microcephaly in humans have identified that CPAP (also named CENPJ) is one of the six genes that influences mammalian brain size. We found that CPAP carries a novel microtubule-destabilizing motif (112 amino acids) that binds to tubulin heterodimers and disassembles preformed microtubules. This 112-residue CPAP directly recognizes the plus end of a microtubule and inhibits microtubule nucleation from the centrosome. However, the molecular mechanism of how CPAP regulates microtubule dynamics and controls brain size is not clear. Interestingly, this 112-residue CPAP carries both an alpha-helical structure and a non-helical structure. To examine the potential role of this alpha-helical structure, we divided this 112-residue CPAP (PN2-3) into two subregions, which contained either the alpha-helical motif (PN2-3N) or the non-helical motif (PN2-3C). Using the in vitro microtubule nucleation assay, our results showed that GST-PN2-3 exhibited the severest constraint on microtubule nucleation from the centrosomes, while GST-PN2-3N and GST-PN2-3C revealed no or only partial suppression, respectively. Together, these results suggest that both the alpha-helical and non-helical structures in PN2-3 are required for full inhibition of microtubule assembling from the centrosomes. A series of proline substitutions, designed to disrupt the putative helix, were also introduced into PN2-3 by site-directed mutagenesis. The proline substitutions dramatically reduce microtubule assembling from the centrosomes, suggesting that the intact alpha-helical structure in PN2-3 is essential for its microtubule-depolymerizing activity. In addition to PN2-3, we defined another polypeptide domain A5N in CPAP that possesses the ability to bind to polymerized microtubules. The binding of A5N polypeptide to polymerized microtubules was confirmed by microtubule sedimentation assay and by transfection of GFP-A5N into mammalian cells. Furthermore, our results showed that CPAP colocalizes with mitotic Aurora-A kinase at the centrosomes throughout the cell cycle and CPAP coimmunoprecipitated with Aurora-A. The in vitro kinase assay demonstrated that CPAP can be phosphorylated by Aurora-A in vitro. Surprisingly, we found that Aurora-A phosphorylation significantly inhibits the microtubule-depolymerizing activity of CPAP. Together, we have dissected the functional domains of CPAP and suggested that Aurora-A may regulate the microtubule-depolymerizing ability of CPAP through phosphorylation.

***Keywords*:** mitosis; microtubule dynaimcs; centrosome; kinase; microtubule depolymerization

Correspondence: Tang K Tang
E-mail: tktang@ibms.sinica.edu.tw

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Involvement of PI-3K and MEK in monocytes/endothelium cells adhesion

Guo Hua Li, Yu-Lin Tu, Zuo Wang, Zhi Sheng Jiang, Zheng Sheng Zuo, Xiang Ou, Chaoke Tang, Guanghui Yi, Yongzong Yang
The Institute of Cardiovascular Disease, Nanhua University, Hengyang, China

This study was to explore whether the signal molecules such as PI-3K,MEK in the SDF-1-CXCR-4 axis participate in monocytes adhesion to enthothelium cells. Cultured endothelial cells ECV-304 were incubated with different concentrations of oxidized low density lipoprotein(ox-LDL).SDF-1α and CXCR-4 mRNA and protein expressions on enthothelium cells and monocytes (THP-1) were determined by Reverse transcript-polymerase chain reaction (RT-PCR) and Western blotting respectively. In ex vivo static adhesion assays , endothelial cells pretreated with ox-LDL were incubated with THP-1 to observe cells adhesion. We found that ox-LDL caused a up-regulation of Endothelial SDF-1 mRNA and protein expressions in a dose-dependent manner. Compared with the control group, adhesion of THP-1 to ECV-304 reached the peak in ox-LDL and SDF-1α group , but was the sharp decline in ox-LDL, PI-3K and MEK inhabitor group. These results show that signal molecules PI-3K,MEK participates in monocytes adhesion to endothelial cells.

***Keywords*:** PI-3K; MEK; SDF-1α; ox-LDL; adhesion

Correspondence: Guohua Li
E-mail: ghli20040219@163.com

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Cloning and characterization of five novel genes encoding kinesin-like proteins in silkworm, *Bombyx mori*

Qiao Wang[1], Huihui Li[1], Wei Zhang[1], Junlin Teng[1], Jianguo Chen[1, 2]
[1]Department of Cell Biology and Genetics, College of Life Sciences, [2]Center for Theoretical Biology, Peking University, Beijing 100871, China

Kinesins are a large family of eukaryotic microtubule-as-

sociated motors, which are reported to be involved in the transport of organelles, protein complexes, and mRNAs. Only one partial sequence of kinesin-like-protein has been cloned and characterized in silkworm, *Bombyx mori*. Here, we report five novel kinesins in *Bombyx mori*, whose predicted proteins range from 482 to 960 amino acids. By sequence analysis, the motor domains and the coiled-coil regions of these five kinesins are identified. Phylogenic analysis categorizes four of them into Kinesin-1, Kinesin-7, Kinesin-13 and Kinesin-14 family respectively, whereas one of them is still considered ungrouped. Moreover, we fused one of them, BmKLPa, with YFP protein using a Bm-NPV-based expression system. BmKLPa colocalizes with microtubules both in BmN cells and silkworms infected by the recombinant BmNPV baculoviruses.

Keywords**:** kinesin; silkworm; motor domain; phylogenesis; baculovirus

Correspondence: Jianguo Chen
E-mail: chenjg@pku.edu.cn

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Type igamma PIP kinase modulates adherens junction and E-cadherin trafficking via a direct interaction with mu1B adaptin

Kun Ling[1], Shawn Bairstow[1], Chateen Carbonara[1], Dmitry Turbin[2], David Huntsman[2], Richard Anderson[1]
[1]Department of Pharmacology, University of Wisconsin Madison, WI 53706, USA; [2]Denetic Pathology Evaluation Centre of the Departments of Pathology, British Columbia Cancer Agency, Vancouver General Hospital and University of British Columbia, Vancouver, Canada

Assembly of E-cadherin-based adherens junction (AJ) is obligatory for establishment of polarized epithelia and plays a key role in repressing the invasiveness of many carcinomas. Here we show that type Igamma phosphatidylinositol phosphate kinase (PIPKIgamma) directly and preferentially interacts with dimeric E-cadherin and controls E-cadherin trafficking by generation of PI4, 5P2. In addition to PI4, 5P2 generation, regulation of trafficking requires that PIPKIgamma act as a scaffold that interacts with and brings together E-cadherin and clathrin adaptor protein (AP) complexes. Deletion of PIPKIgamma sequences required for AP complexes binding resulted in a loss of E-cadherin transport to the plasma membrane and AJ assembly. The association of PIPKIgamma with E-cadherin is also required. An E-cadherin germline mutation, which does not form AJs and is correlated with hereditary gastric cancer, lost PIPKIgamma binding and showed disrupted basal-lateral membrane targeting. These results provide a mechanism underlying the spatial-temporal generation of PI4, 5P2 in trafficking events and support a novel cargo assembly model for E-cadherin trafficking.

Keywords**:** E-cadherin; PI4, 5P2; phosphatidylinositol-4-phosphate 5-kinase gamma; clathrin adaptor complex; trafficking

Correspondence: Kun Ling
E-mail: kunling@wisc.edu

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Dynamics of actin and myosin II filaments, generation of force, and signals in chemotaxis of *Dictyostelium discoideum*

Shigehiko Yumura, Md Kamuruzzaman Plamanik, Yoshiaki Iwadate
Deapartment of Biology, Faculty of Science, Yamaguchi University, Yamaguchi, Japan

TIRF microscopy reveals dynamics of actin and myosin II filaments Actin and myosin II filaments were simultaneously observed in chemotactic *Dictyostelium* cells expressing EGFP-actin-binding domain of ABP 120 and mRFP-myosin heavy chain. Actin filaments were newly formed on the cell membrane and myosin II attached to the actin filaments as the cell moved forward. Interestingly, the newly formed actin filaments did not move relatively to the substratum. Myosin filaments detached from the actin filaments with a frequency of about 7 s but did not move relatively to the substratum during the attachment. Since both did not move relatively to the substratum, they concentrated toward the rear of the cell as it moved forward. There must be a possible mechanism for releasing and recycling these elements from the rear because the amount of both proteins there was almost constant. Regarding myosin II, detachment of filaments from the actin can be explained by disassembly of the filaments. This disassembly is mediated by the phosphorylation of myosin heavy chains. Actually, 3xALA myosin, in which the 3 phosphorylatable threonine residues are converted to alanine residues, over-accumulated at the rear of the migrating cell. Next, we observed dynamics of 3xALA myosin in live cells. Interestingly, 3xALA myosin revealed slower turnover, which can explain its over-accumulation at the rear. Force microscopy during chemotaxis to investigate the mechanism how the myosin gives the force for cell migration, the force of the cell was measured by flexible silicone substratum and mapped in pseudocolor. Myosin-null cells showed less traction force at the tip of pseudopods than Wild-type cells. We are now observing simultaneously myosin and the force. Signals for myosin II PTEN is a tumor suppressor gene, which helps regulate the cycle of cell division and cell polarity. Previous researches showed similar localization between

PTEN and myosin. Therefore, we compared the localization of both proteins in live cells expressing EGFP-PTEN and mRFP-myosin. PTEN and myosin II localized at the rear of migrating cells. When a cell changed the direction, PTEN appeared at the retracting pseudopodes and then myosin II accumulated at the same place. Therefore, PTEN may dictate the localization of myosin II.

***Keywords*:** actin; myosin; TIRF microscopy

Correspondence: Shigehiko Yumura
E-mail: yumura@yamaguchi-u.ac.jp

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Dynamic localization of Ca^{2+}-calmodulin signaling system and the essential role in the hyphal tip growth and conidiation in *Aspergillus nidulans*

Shaochun Chen, Gang Wang, Qi Guan, Ling Lu
College of Life Science, Nanjing Normal University, Nanjing, China

The calmodulin (CaM) localization pattern in the growing hyphal tip of Aspergillus nidulans was studied with the functional GFP::CaM fusion protein by extra-integrated expression plasmid. A faint tip-high gradient of CaM was found in the growing hyphal tip, with CaM highly localized in the region corresponding to the Spitzenkörper forming a bright granule. The position of highly concentrated CaM in the extreme apex seemed to determine the orientation of the hypha. The normal pattern of CaM localization was also shown to be dependent on the integrated actin cytoskeleton. When the growth of the hyphal tip ceased, CaM failed to localize in the bright granule and was evenly distributed in the hyphal tip. In addition, The homologous integration strain that CaM was replaced by alcA-GFP:: CaM was constructed successfully. When the expression of CaM was turned off on the conditional repression medium, the hyphal growth was ceased, the severe defective mini-colonies were formed. These findings suggest that CaM may play an important role in establishing and maintaining apical organization, morphogenesis, and growth in *Aspergillus nidulans*.

***Keywords*:** *Aspergillus nidulans*; calmodulin; tip growth; cytoskeleton; Spitzenkörper

Correspondence: Ling Lu
E-mail: linglu@njnu.edu.cn

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IP3KB is a negative regulator in neutrophil chemotaxis

Yonghui Jia[1], K K Subramanian[1], Christophe Erneux[2], Hongbo Luo[1]
[1]Chidren's Hospital Boston, Harvard Medical School, Boston 02115, USA; [2]Free University of Brussels, Brussels B-1070, Belgium

Regulation of neutrophil chemotaxis is critical in inflammatory responses. While improper chemotaxis can lead to decreased neutrophil recruitment and lack of a response, enhanced chemotaxis and recruitment of neutrophils can lead to tissue damage. During chemotaxis, G-protein coupled receptor mediated PI3K/PI(3, 4, 5)P_3 production elicits plasma membrane translocation of PH domain containing proteins. This signaling event has been shown to be essential for neutrophil chemotaxis. In this study, we have defined a novel pathway regulating this event. We demonstrate that InsP4 can compete with PI(3, 4, 5)P_3 for PH domain binding. IP3 kinase (IP3K) phosphorylates inositol (1, 4, 5) triphosphate (InsP3) to produce inositol (1, 3, 4, 5) tetraphosphate (InsP4). We show that IP3KB is the major IP3K isoforms in neutrophils. Supporting our hypothesis, we observed that IP3KB null neutrophils show increased sensitivity to fMLP stimulation. While the migration speed of IP3KB null neutrophils is normal, the directionality towards a point source of chemoattractant is attenuated. Our results are consistent with a hypothesis that IP3KB is a negative regulator of neutrophil chemotaxis. The mechanisms for this negative regulation are currently under investigation.

***Keywords*:** neutrophil chemotaxis; IP3KB

Correspondence: Yonghui Jia
E-mail: yonghui.jia@childrens.harvard.edu

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Regulation of the disassembly of the microtubule-based cellular organelle - cilia/flagella

Junmin Pan
Department of Biological Sciecnes and Biotechnology, Tsinghua University, Beijing 10084, China

Cilia/flagella are disassembled prior to cell division, during cell differentiation and in response to environmental stress. In Chlamydomonas, cells employ two pathways to disassemble their flagella: gradual, progressive disassembly of the axoneme at the flagellar tip leading to complete flagellar resorption and rapid, highly localized disassembly of the axoneme at a site near the transition zone leading to flagellar detachment or shedding. I am interested in understanding the molecular mechanisms that underlie these processes. I showed that -the aurora-like protein kinase CALK is a regulator common to both disassembly pathways: In both pathways, CALK is phosphorylated, activated and essential. I have also found that flagellar resorption is independent of the LF4-mediated flagellar length control mechanism.

Flagella in lf4 mutant were resorbed normally and CALK phosphorylation was undisrupted. Moreover, when flagellar disassembly is triggered, axonemal disassembly, evidenced by a 2-4 fold increase of disassembled flagellar components in the flagellar matrix, is stimulated and concomitantly, trafficking of intraflagellar transport (IFT) particles and motors is increased several-fold. Importantly, the newly entering IFT particles are without cargo, thereby providing a source of empty binding sites on the particles ready to retrieve the disassembled flagellar components from the tip back to the cell body.

***Keywords*:** intraflagellar transport; cilia and flagella; protein kinase; chlamydomonas

Correspondence: Junmin Pan
E-mail: swxrs@mail.tsinghua.edu.cn

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ABCG2 in drug resistance and tumorigenesis

Lei Du, Quan Chen
State Key Lab of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

Cancer stem cells are a minor population of cancer cells that may play an important role in tumorigenesis and cancer relapse after chemotherapy. The existence of cancer stem cells has been identified in human leukemia and solid tumors including lung cancer, prostate cancer, breast cancer and brain cancer. Cancer stem cell has normal stem cell properties such as self-renewal and differentiation. It is also related to cancer drug resistance and metastatic properties. We focused on side population which has been used as surrogate marker for cancer stem cell. In our study, we over-expressed ABCG2 transporter protein and its truncate in three kinds of colon cancer cell lines, which confers the SP phenotype. The GFP tagged ABCG2 located at plasma membrane which enabled cells to have higher drug resistance. However, over-expression of ABCG2 didn't impact on cell cycle and colony formation activity *in vitro*. We also examined the tumorigenesis of ABCG2 over-expressing cells *in vivo*. 1×10^6 cells were injected into 6-week-old female nude mice. The mice were sacrificed when half of the body weight in any groups was sharply decreased. The tumors were removed from mice and weighed, and then immunofluorescence was performed to identify the origin of the tumor cells. Much to our surprise, ABCG2 over-expressing cells failed to generate bigger tumors. Our data suggest that ABCG2 may play a significant role in drug resistance, while it has little effects on tumorigenesis, and the reason is being discussed.

***Keywords*:** ABCG2; drug resistance; tumorigenesis

Correspondence: Quan Chen
E-mail: chenq@ioz.ac.cn

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A role of estrogen in the ultrastructure and lymphatic absorption of the stomata in mouse ovarian bursa

Meng Li[1], Tianhua Zhou[2], Yun Gao[1], Jicheng Li[1]
[1]Institute of Cell Biology and Tissue Engineering, [2]Institute of Medical Genetics, Zhejiang University School of Medicine, Hangzhou, China

The mouse ovarian bursa required for ovulation is a key player in maintaining normal ovarian microenvironment. The lymphatic stomata are believed as a major contributor to execute the function of mouse ovarian bursa, whereas little is known about their ultrastructure and regulation. Here, we examined the lymphatic stomata of mouse ovarian bursa by scanning electron microscopy (SEM) and transmission electron microscopy (TEM), and investigated its regulation by estrogen. We found that the mesothelium on the parietal ovarian bursa of the mouse was composed of the cuboidal and flattened cells. The lymphatic stomata with round, oval and even anomalous shapes were mainly among the cuboidal cells. The particles, cells and fluid passed through the stomata and entered into the lymphatic drainage unit composed of the connective tissue and the lymphatic endothelial cells beneath the stomata. We also employed trypan blue as a tracer and found that the absorption through lymphatic stomata was increased by estrogen that enlarged the average opening area of lymphatic stomata. Furthermore, we detected that there existed estrogen receptors in the nuclei of the mesothelial cells on the inner layer of the ovarian bursa by using immuno-electron microscopy. Taken together, these data suggest that both the absorption and opening area of the lymphatic stomata of mouse ovarian bursa may be influenced by estrogen.

***Keywords*:** lymphatic stomata; ovary bursa ; estrogen receptor; ultrastructure; mouse

Correspondence: Meng Li
E-mail: lmbcwan@163.com