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ダイジェスト

JB SPECIAL ISSUE—MULTIFACETED PROTEIN WORLD

GUEST EDITORS: HIDEKI TAGUCHI AND RICHARD I. MORIMOTO JB SPECIAL ISSUE—REVIEW

Nascent chain-mediated translation regulation in bacteria: translation arrest and intrinsic ribosome destabilization

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Proteins that exsert physiological functions during being translated have been discovered from prokaryotes to eukaryotes. These proteins, also called regulatory nascent chains, are common in interacting co-translationally with the ribosomes to stall them. In most cases, such a translational arrest is induced or released in response to changes in the intracellular environment. Cells take advantage of such an environmental sensitivity as a sensor to feedback-regulate gene expression. Recent studies reveal that certain nascent chains could also destabilize the translating ribosomes, leading to stochastic premature translation termination. In this review, we introduce several examples of bacterial nascent chain-based mechanisms of translation regulation by which bacteria regulate cellular functions.

Keywords: Translation arrest, ribosome, stalling, IRD, arrest peptide

Identification of unannotated coding sequences and their physiological functions

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Most protein-coding sequences (CDSs) are predicted sequences based on criteria such as a size sufficient to encode a product of at least 100 amino acids and with translation starting at an AUG initiation codon. However, recent studies based on ribosome profiling and mass spectrometry have shown that several RNAs annotated as long as noncoding RNAs are actually translated to generate polypeptides of fewer than 100 amino acids and that many proteins are translated from near-cognate initiation codons such as CUG and GUG. Furthermore, studies of genetically engineered mouse models have revealed that such polypeptides and proteins contribute to diverse physiological processes. In this review, we describe the latest methods for the identification of unannotated CDSs and provide examples of their physiological functions.

Keywords: long noncoding RNA (lncRNA), near-cognate initiation codon, polypeptide, ribosome profiling, translation

In situ digestion of alcohol-fixed cells for quantitative proteomics

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Currently, the bottom-up approach, in which proteins are digested by enzymes such as trypsin prior to mass spectrometry, is the mainstream approach in mass spectrometer-based proteomics. In this approach, the enzymatic digestion process strongly affects the reproducibility of protein identification and quantification. Here, we quantitatively evaluated the enzymatic digestion of proteins under various conditions by quantitative proteomics using data-independent acquisition and found that proteins precipitated with acetone after solubilization with SDS were fully digestible without re-solubilization. This result implies that organic solvent treatment makes cells amenable to trypsin digestion. Direct trypsin digestion of methanol-fixed cells achieved the same digestion efficiency and quantitative reproducibility as the conventional method. Furthermore, this method was found to be equally applicable to mouse liver samples. The establishment of this method indicates that the sample preparation process in bottom-up proteomics can be simplified while maintaining high digestion efficiency and is expected to become a general method for sample preparation in bottom-up proteomics in the future.

Keywords: mass spectrometry, proteomics, sample preparation, trypsin

Abbreviations: AGC, auto gain control; Ambic, ammonium bicarbonate; BCA, bicinchoninic acid; CVs, coefficients of variation; DDA, data-dependent acquisition; DIA, data-independent acquisition; DMEM, Dulbecco's modified Eagle's medium; FBS, foetal bovine serum; GdnHCl, guanidine hydrochloride; IAA, iodoacetamide; iSDAC, in situ digestion of alcohol-fixed cells; IT, injection times; lys-C, lysyl endopeptidase; LC, liquid chromatography; MS, mass spectrometry; PBS, phosphate-buffered saline; SDC, sodium deoxycholate; SLS, sodium deoxycholate-N-lauroylsarcosinate; TCA, trichloroacetic acid; TCEP, tris (2-carboxyethyl) phosphine hydrochloride

Classification of proteins inducing liquid–liquid phase separation: sequential, structural and functional characterization

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Liquid–liquid phase separation (LLPS) within the cell can form biological condensates, which are increasingly recognized to play important roles in various biological processes. Most proteins involved in LLPS are known to be intrinsically disordered proteins containing intrinsically disordered regions (IDRs) with low complexity regions (LCRs). The proteins driving LLPS were selected from databases of LLPS-related proteins and then classified into three classes according to the components in the condensates. Through *in silico* analyses, we found that proteins in the homo class, those that induce LLPS without partner molecules, have different IDRs and LCRs compared with the reference proteome. In contrast, proteins in the other classes, those that induce LLPS with partner proteins (the hetero class) or nucleic acids (the mixed class), did not show a clear difference to the reference proteome in IDRs and LCRs. The hetero-class proteins contained structural domains to serve protein–protein interactions, and the mixed-class ones had the structural domains associated with nucleic acids. These results suggest that IDRs in the homo-class proteins have unique IDRs, which provide multivalent interaction sites required for LLPS, whereas the hetero- and mixed-class proteins can induce LLPS through the combination of the interaction among LCRs, structural domains and nucleic acids.

Keywords: sequence analysis, liquid–liquid phase transition, intrinsically disordered proteins, bioinformatics<bioenergetics, amino acid composition

Proofreading of protein localization mediated by a mitochondrial AAA-ATPase Msp1

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Normal cellular functions rely on correct protein localization within cells. Protein targeting had been thought to be a precise process, and even if it fails, the mistargeted proteins were supposed to be quickly degraded. However, this view is rapidly changing. Tail-anchored (TA) proteins are a class of membrane proteins that possess a single transmembrane domain (TMD) near the C-terminus and are post translationally targeted to the endoplasmic reticulum (ER) membrane, mitochondrial outer membrane (OM), and peroxisomal membrane, yet they can be mistargeted to the mitochondrial OM. The mistargeted TA proteins can be extracted from the OM by a mitochondrial AAA-ATPase Msp1/ATAD1 and transferred to the ER. If they are regarded as aberrant by the ER protein quality control system, they are extracted from the ER membrane for proteasomal degradation in the cytosol. If they are not regarded as aberrant, they are further transported to downstream organelles or original destinations along the secretory pathway. Thus, Msp1 contributes to not only degradation but also “proofreading” of the targeting of mislocalized TA proteins.

Keywords: tail-anchored proteins, proofreading, Msp1, mistargeting, GET pathway

Repeat-associated non-AUG translation in neuromuscular diseases: mechanisms and therapeutic insights

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Expanded short tandem repeats cause more than 50 monogenic diseases, which are mostly neuromuscular diseases. In the non-coding repeat expansion diseases, in which the expanded repeat sequence is located outside of the coding region, the toxicity of the transcribed repeat-containing RNAs had been the focus of research. However, recent studies have revealed that repeat RNAs can be translated into repeat polypeptides, despite the lack of an AUG initiation codon, by non-canonical repeat-associated non-AUG translation (RAN translation). RAN translated repeat polypeptides have actually been confirmed in patients' tissues. Moreover, various cellular and animal disease models have demonstrated the toxicity of these peptides, suggesting the pathogenic roles of RAN translation in the repeat expansion diseases. In this review, we will outline RAN translation, from the viewpoint of its molecular mechanisms to its potential as a therapeutic target for the repeat expansion diseases.

Keywords: RAN translation, repeat expansion disease, non-canonical translation, neuromuscular disease, neurodegeneration.

REGULAR PAPER
BIOCHEMISTRY
Lipid Biochemistry

Identification and characterization of bioactive metabolites of 12-hydroxyheptadecatrienoic acid, a ligand for leukotriene B4 receptor 2

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12(*S*)-hydroxyheptadecatrienoic acid (12-HHT) is a bioactive fatty acid synthesized from arachidonic acid via the cyclooxygenase pathway and serves as an endogenous ligand for the low-affinity leukotriene B_4 receptor 2 (BLT2). Although the 12-HHT/BLT2 axis contributes to the maintenance of epithelial homeostasis, 12-HHT metabolism under physiological conditions is unclear. In this study, 12-keto-heptadecatrienoic acid (12-KHT) and 10,11-dihydro-12-KHT (10,11dh-12-KHT) were detected as 12-HHT metabolites in the human megakaryocytic cell line MEG01s. We found that 12-KHT and 10,11dh-12-KHT are produced from 12-HHT by 15-hydroxyprostaglandin dehydrogenase (15-PGDH) and prostaglandin reductase 1 (PTGR1), key enzymes in the degradation of prostaglandins, respectively. The 15-PGDH inhibitor SW033291 completely suppressed the production of 12-KHT and 10,11dh-12-KHT in MEG01s cells, resulting in a 9-fold accumulation of 12-HHT. 12-KHT and 10,11dh-12-KHT were produced in mouse skin wounds, and the levels were significantly suppressed by SW033291. Surprisingly, the agonistic activities of 12-KHT and 10,11dh-12-KHT on BLT2 were comparable to that of 12-HHT. Taken together, 12-HHT is metabolized into 12-KHT by 15-PGDH, and then 10,11dh-12-KHT by PTGR1 without losing the agonistic activity.

Keywords: prostaglandins, BLT2, arachidonic acid, 15-PGDH, 12-HHT

Enzymology

Characterization of the enzymatic properties of human RNPEPL1/aminopeptidase Z

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It is now evident that the M1 family of aminopeptidases play important roles in many pathophysiological processes. Among them, the enzymatic properties of arginyl aminopeptidase-like 1 (RNPEPL1) are characterized only by its truncated form. No peptide substrate has been identified. To characterize the enzymatic properties of RNPEPL1 in more detail, the full-length protein was expressed in *Escherichia coli* and purified to homogeneity. The full-length RNPEPL1 showed rather restricted substrate specificity and basic amino acid preference towards synthetic substrates, which was different from the previously reported specificity characterized by the truncated form. Searching for peptide substrates, we found that several peptides, such as Met-enkephalin and kallidin, were cleaved. RNPEPL1 cleaved bradykinin to de-[Arg]-bradykinin despite the presence of proline at the P2′-position. The enzyme cleaved Met-enkephalin but not dynorphin $A_{1–17}$. Similar to aminopeptidase B, the full-length RNPEPL1 showed basic amino acid preference towards both synthetic and peptide substrates. In addition to the unusual cleavage of bradykinin, this enzyme shows chain length-dependent cleavage of peptide substrates sharing N-terminal amino acid sequence. This is the first study to report the enzymatic properties of the full-length human RNPEPL1 as an aminopeptidase enzyme.

Keywords: substrate specificity, RNPEPL1, peptide substrate, enzymatic property, aminopeptidase

MOLECULAR BIOLOGY
Molecular Biology General

Structural insight into the recognition of the linear ubiquitin assembly complex by Shigella E3 ligase IpaH1.4/2.5

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Pathogenic bacteria deliver virulence factors called effectors into host cells in order to facilitate infection. The *Shigella* effector proteins IpaH1.4 and IpaH2.5 are members of the 'novel E3 ligase' (NEL)-type bacterial E3 ligase family. These proteins ubiquitinate the linear ubiquitin assembly complex (LUBAC) to inhibit nuclear factor (NF)-κB activation and, concomitantly, the inflammatory response. However, the molecular mechanisms

underlying the interaction and recognition between IpaH1.4 and IpaH2.5 and LUBAC are unclear. Here we present the crystal structures of the substrate-recognition domains of IpaH1.4 and IpaH2.5 at resolutions of 1.4 and 3.4 Å, respectively. The LUBAC-binding site on IpaH1.4 was predicted based on structural comparisons with the structures of other NEL-type E3s. Structural and biochemical data were collected and analysed to determine the specific residues of IpaH1.4 that are involved in interactions with LUBAC and influence NF-κB signaling. The new structural insight presented here demonstrates how bacterial pathogens target innate immune signaling pathways.

Keywords: ubiquitin ligase, Shigella flexneri, effector, crystal structure

CELL

Receptors and Signal Transduction

Indole-derived compound SIS3 targets a subset of activated Smad complexes

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Smad2 and Smad3 are receptor-regulated Smad proteins that transmit signals from cytokines belonging to the transforming growth factor (TGF)-β family, which are vital for adult tissue homeostasis. The overactivation of such proteins often engenders the development of pathological conditions. Smad3 reportedly mediates TGF-β–induced fibrosis. Although various potential Smad3-specific inhibitors are being developed, their specificity and action mechanisms remain largely unknown. This study aimed to establish a biochemical platform to monitor Smad2- or Smad3-dependent TGF-β signaling using *SMAD2*, *SMAD3* and *SMAD2/3* knockout cell lines alongside TGF-β–dependent luciferase reporters and Smad mutant proteins. Using this platform, SIS3, an indole-derived compound widely used as a specific Smad3 inhibitor, was observed to preferentially suppress a subset of activated Smad complexes. However, its inhibition did not favor Smad3 signaling over Smad2 signaling. These findings indicate that SIS3 can be employed as a probe to examine the heterogeneous nature of Smad signaling that induces gene expression. However, its use as a Smad3-specific inhibitor should be avoided.

Keywords: transcription, TGF-β, Smad3, SIS3, pharmacological inhibitor

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ダイジェスト

JB COMMENTARY

Chondroitin sulfate glycosaminoglycans function as extra/pericellular ligands for cell surface receptors

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Chondroitin sulfate (CS) chains, a class of sulfated glycosaminoglycan (GAG) polysaccharides, are ubiquitously distributed in extra/pericellular matrices that establish microenvironmental niches to support a multitude of cellular events. Such wide-ranging functions of CS chains are attributable not only to their sulfation pattern-dependent structural divergence, but also to their multiple modes of action. Although it has long been accepted that CS chains act as passive structural scaffolds that often behave as co-receptors and/or reservoirs for various humoral factors, the discovery of cell surface receptor molecules for distinct CS chains has offered insights into a novel mode of CS function as dynamic extra/pericellular signaling ligands. A recent report by Gong *et al.* (Identification of PTPRσ-interacting proteins by proximity-labeling assay. *J. Biochem*. 2021; 169: 187–194) also strongly reinforced the physiological importance of CS receptor-mediated signaling pathways. In this commentary, we briefly introduce the functional aspects of CS chains as extra/pericellular signaling molecules.

Keywords: sulfation, proteoglycan, glycosaminoglycan, CS receptor, chondroitin sulfate

Inhibitory machinery for the functional dystroglycan glycosylation

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Dystroglycan (DG), a muscular transmembrane protein, plays a critical role in transducing extracellular matrix-derived signals to the cytoskeleton and provides physical strength to skeletal muscle cell membranes. The extracellular domain of DG, α-DG, displays unique glycosylation patterns. Fully functional glycosylation is required for this domain to interact with components of extracellular matrices, including laminin. One of the unique sugar compositions found in such functional glycans on DG is

two ribitol phosphates that are transferred by the sequential actions of fukutin (FKTN) and fukutin-related protein (FKRP), which use CDP-ribitol as a donor substrate. These are then further primed for matriglycan biosynthesis. A recent *in vitro* study reported that glycerol phosphate could be similarly added to *α*-DG by FKTN and FKRP if they used CDP-glycerol (CDP-Gro) as a donor substrate. However, the physiological relevance of these findings remains elusive. Imae et al. addressed the knowledge gap regarding whether CDP-Gro is present in mammals and how CDP-Gro is synthesized and functions in mammals.

Keywords: ribitol phosphate, PCYT2, glycerol phosphate, dystroglycan, CDP-Gro synthase

RAPID COMMUNICATION

Reactive oxygen species are associated with the inhibitory effect of *N*-(4-hydroxyphenyl)-retinamide on the entry of the severe acute respiratory syndrome-coronavirus 2

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N-(4-hydroxyphenyl)-retinamide (4-HPR) inhibits the dihydroceramide Δ4-desaturase 1 (DEGS1) enzymatic activity. We previously reported that 4-HPR suppresses the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) spike protein-mediated membrane fusion through a decrease in membrane fluidity in a DEGS1-independent manner. However, the precise mechanism underlying the inhibition of viral entry by 4-HPR remains unclear. In this study, we examined the role of reactive oxygen species (ROS) in the inhibition of membrane fusion by 4-HPR because 4-HPR is a well-known ROS-inducing agent. Intracellular ROS generation was found to be increased in the target cells in a cell–cell fusion assay after 4-HPR treatment, which was attenuated by the addition of the antioxidant, *α*-tocopherol (TCP). The reduction in membrane fusion susceptibility by 4-HPR treatment in the cell–cell fusion assay was alleviated by TCP addition. Furthermore, fluorescence recovery after photobleaching analysis showed that the lateral diffusion of glycosylphosphatidylinositol-anchored protein and SARS CoV-2 receptor was reduced by 4-HPR treatment and restored by TCP addition. These results indicate that the decrease in SARS-CoV-2 spike protein-mediated membrane fusion and membrane fluidity by 4-HPR was due to ROS generation. Taken together, these results demonstrate that ROS production is associated with the 4-HPR inhibitory effect on SARS-CoV-2 entry.

Keywords: TCP, SARS-CoV-2, ROS, 4-HPR

REGULAR PAPER BIOCHEMISTRY

Metabolism and Bioenergetics

PPAR*α* activation partially drives NAFLD development in liver-specific Hnf4a-null mice

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HNF4*α* regulates various genes to maintain liver function. There have been reports linking HNF4*α* expression to the development of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis. In this study, liver-specific *Hnf4a*-deficient mice (*Hnf4a*$^{\Delta Hep}$ mice) developed hepatosteatosis and liver fibrosis, and they were found to have difficulty utilizing glucose. In *Hnf4a*$^{\Delta Hep}$ mice, the expression of fatty acid oxidation-related genes, which are PPAR*α* target genes, was increased in contrast to the decreased expression of PPAR*α*, suggesting that *Hnf4a*$^{\Delta Hep}$ mice take up more lipids in the liver instead of glucose. Furthermore, *Hnf4a*$^{\Delta Hep}$/*Ppara*$^{-/-}$ mice, which are simultaneously deficient in HNF4*α* and PPAR*α*, showed improved hepatosteatosis and fibrosis. Increased C18:1 and C18:1/C18:0 ratio was observed in the livers of *Hnf4a*$^{\Delta Hep}$ mice, and the transactivation of PPAR*α* target gene was induced by C18:1. When the

C18:1/C18:0 ratio was close to that of *Hnf4a*$^{\Delta Hep}$ mouse liver, a significant increase in transactivation was observed. In addition, the expression of *Pgc1a*, a coactivator of PPARs, was increased, suggesting that elevated C18:1 and *Pgc1a* expression could contribute to PPARα activation in *Hnf4a*$^{\Delta Hep}$ mice. These insights may contribute to the development of new diagnostic and therapeutic approaches for NAFLD by focusing on the HNF4α and PPARα signaling cascade.

Keywords: Fatty acid, Lipid, Liver, Nuclear receptor, Steatohepatitis

Reactive Oxygen and Nitrogen Species

Formation of hierarchical assemblies by collagen peptides derived from fish skin and bladder and their subsequent application as antiperoxide agents in lipid-rich food

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This study attempts to identify the significant role played by the secondary and tertiary structure of collagen-derived peptides that are involved in lipid peroxide quenching in food products. Fish collagen hydrolysate (CH) was extracted with an efficiency of 70%. The constituent peptides of CH (8.2–9.7 kDa) existed in a polyproline-II (PP-II) conformation and at a minimum concentration of 1 mg ml^{-1} and pH range 7 to 8, assembled into a stable, hierarchical, quasi-fibrillar (QF) network. The peroxide quenching activity of this QF-CH increased with increasing ionic stability of the assembly and decreased upon proteolytic dismantling. Upon being used as an additive, the QF-CH reduced peroxide formation by 84.5% to 98.9% in both plant and fish-based oil and increased the shelf life of soya oil by a factor of 5 after 6 months of storage. The addition of QF-CH to cultured cells quenched peroxide ions generated *in situ* and decreased stressor activity by a factor of 12.16 abundant peptides were identified from the CH. The reason behind the high efficacy displayed by CH was attributed to its unique charge distribution, prevalence of proton-donating amino acid residues and proximal charge delocalization by the QF network, making fish derived CH a suitable substitute for antiperoxide agents in lipid-rich food.

Keywords: structure–activity relation, self-assembly, peroxidation, Hydrolysate, collagenpeptide

Analytical Biochemistry

An Assay Method for Characterizing Bacteriophage T7 RNA Polymerase Activity by Transcription–Translation (TX–TL) System

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T7 system is a commonly used in protein expression and the highest transcription activity of T7 RNAP usually caused the instability of T7 system. In order to apply T7 system extensively, it is essential to characterize T7 RNAP activity. In the present paper, an assay method for T7 RNAP activity was developed with a transcription–translation (TX–TL) system. After the optimization of TX–TL system, the operating parameters were determined as 34°C, 60 min with 20 ng/μl of plasmid DNA template. The standard curve of TX–TL assay method indicated an excellent correlation (r=0.998), and the sensitivity was better than that of western blotting method. The precision investigation indicated a mean-relative error of 2.58% and a standard-relative error of 7.01%. Moreover, the cell lysate could be added directly to the optimized TX–TL system without affecting T7 RNAP activity assay. The feasibility of present method was further confirmed by characterizing T7 RNAP activity in cell lysate of five strains of *Escherichia coli* (*E. coli*) DH5α with different T7 RNAP activities and seven commercial strains of *E. coli* (DE3). The present assay method for T7 RNAP activity would have a great application in synthetic biology, metabolic engineering, enzyme engineering and biomedicine.

Keywords: transcription–translation system (TX–TL), T7 RNA Pactivity, expression system, cell lysate, assay method

MOLECULAR BIOLOGY

Molecular Biology General

Klotho protects chromosomal DNA from radiation-induced damage

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Klotho is an anti-aging, single-pass transmembrane protein found

mainly in the kidney. Although aging is likely to be associated with DNA damage, the involvement of Klotho in protecting cells from DNA damage is still unclear. In this study, we examined DNA damage in human kidney cells and mouse kidney tissue after ionizing radiation (IR). The depletion and overexpression of Klotho in human kidney cells reduced and increased the cell survival rates after IR, respectively. The formation of γ-H2AX foci, representing DNA damage, was significantly elevated immediately after IR in cells with Klotho depletion and decreased in cells overexpressing Klotho. These results were confirmed in mouse renal tissues after IR. Quantification of DNA damage by a comet assay revealed that the Klotho knockdown significantly increased the amount of DNA damage immediately after IR, suggesting that Klotho protects chromosomal DNA from the induction of damage, rather than facilitating DNA repair. Consistent with this notion, Klotho was detected in both the nucleus and cytoplasm. In the nucleus, Klotho may serve to protect chromosomal DNA from damage, leading to its anti-aging effects.

Keywords: aging, DNA damage, ionizing radiation, Klotho, γ-H2AX

CELL

Biomembranes, Organelles and Protein Sorting Jaw1/LRMP is associated with the maintenance of Golgi ribbon structure

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Jaw1/LRMP is a membrane protein that is localized to the endoplasmic reticulum and outer nuclear membrane. Previously, we revealed that Jaw1 functions to maintain nuclear shape by interacting with microtubules as a Klarsicht/ANC-1/Syne/homology (KASH) protein. The loss of several KASH proteins causes defects in the position and shape of the Golgi apparatus as well as the nucleus, but the effects of Jaw1 depletion on the Golgi apparatus were poorly understood. Here, we found that siRNA-mediated Jaw1 depletion causes Golgi fragmentation with disordered ribbon structure in the melanoma cell, accompanied by the change in the localization of the Golgi-derived microtubule network. Thus, we suggest that Jaw1 is a novel protein to maintain the Golgi ribbon structure, associated with the microtubule network.

Keywords: microtubule network, LINC complex, KASH protein, Jaw1/LRMP, Golgi apparatus

Abbreviations: Ac-Tubulin, Acetylated-Tubulin; CLASPs, CLIP-associated proteins; DNase I, Deoxyribonuclease I; ER, Endoplasmic reticulum; GM130, Golgi matrix protein 130 kDa; GRASP65, Golgi reassembly-stacking protein 1; GRASP55, Golgi reassembly-stacking protein 2; HRP, Horseradish peroxidase; ITPR, Inositol 1,4,5-trisphosphate receptor; KASH, Klarsicht/ANC-1/Syne/homology; KD, knockdown; KIF20A, Kinesin-like protein KIF20A; LINC, Linker of nucleus and cytoskeletons; LRMP, Lymphoid restricted membrane protein; MTCL1, Microtubule cross-linking factor 1; NT, Non-targeting; ONM, outer nuclear membrane; siRNA, short interfering RNA; SUN, Sad-1/UNC-84; TEM, transmission electron microscopy