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ダイジェスト

JB SPECIAL ISSUE—NUCLEOSKELETON-ORIENTED NUCLEAR ARCHITECTURE AND DYNAMICS TO REGULATE GENOME FUNCTIONS GUEST EDITORS: MASAHIKO HARATA, KEI MIYAMOTO AND PIERGIORGIO PERCIPALLE

JB Special Issue—Commentary

Nucleoskeleton proteins for nuclear dynamics

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The eukaryotic nucleus shows organized structures of chromosomes, transcriptional components and their associated proteins. It has been believed that such a dense nuclear environment prevents the formation of a cytoskeleton-like network of protein filaments. However, accumulating evidence suggests that the cell nucleus also possesses structural filamentous components to support nuclear organization and compartments, which are referred to as nucleoskeleton proteins. Nucleoskeleton proteins including lamins and actin influence nuclear dynamics including transcriptional regulation, chromatin organization and DNA damage responses. Furthermore, these nucleoskeleton proteins play a pivotal role in cellular differentiation and animal development. In this commentary, we discuss how nucleoskeleton-based regulatory mechanisms orchestrate nuclear dynamics.

Keywords; lamin, nuclear actin, nuclear dynamics, nucleoskeleton

JB Special Issue—Reviews

A dynamic actin-dependent nucleoskeleton and cell identity

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Actin is an essential regulator of cellular functions. In the eukaryotic cell nucleus, actin regulates chromatin as a *bona fide* component of chromatin remodelling complexes, it associates with nuclear RNA polymerases to regulate transcription and is involved in co-transcriptional assembly of nascent RNAs into ribonucleoprotein complexes. Actin dynamics are, therefore, emerging as a major regulatory factor affecting diverse cellular processes. Importantly, the involvement of actin dynamics in nuclear functions is redefining the concept of nucleoskeleton from a rigid scaffold to a dynamic entity that is likely linked to the three-dimensional organization of the nuclear genome. In this review, we discuss how nuclear actin, by regulating chromatin structure through phase separation may contribute to the architecture of the nuclear genome during cell differentiation and facilitate the expression of specific gene programs. We focus specifically on mitochondrial genes and how their dysregulation in the absence of actin raises important questions about the role of cytoskeletal proteins in regulating chromatin structure. The discovery of a novel pool of mitochondrial actin that serves as 'mitoskeleton' to facilitate organization of mtDNA supports a general role for actin in genome architecture and a possible function of distinct actin pools in the communication between nucleus and mitochondria.

Keywords; chromatin and transcription regulation, development and differentiation, genome organization and integrity, mitochondria, nuclear actin

The dynamics of the nuclear environment and their impact on gene function

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In the last decades, it has become increasingly clear how the modulation of spatial organization of chromatin over time and through the cell cycle is closely connected to gene function regulation. Different physicochemical stimuli contribute to the realization of specific transcriptional programs and finally to a specific cellular phenotype. In this review, we aim to describe the current knowledge about the dynamics regulating the movements and the interactions of molecules within the nucleus and their impact on gene functions. In particular, taking into account that these forces exert their effect in a nuclear environment characterized by a high concentration of molecules, we will discuss the role of proteins and structures that regulate these movements and transduce physicochemical signals acting on the cell to the nucleus.

Keywords; chromatin, molecular crowding, nuclear architecture, nuclear physicochemical dynamics, phase separation

The roles of nuclear myosin in the DNA damage response

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Myosin within the nucleus has often been overlooked due to their importance in cytoplasmic processes and a lack of investigation. However, more recently, it has been shown that their nuclear roles are just as fundamental to cell function and survival with roles in transcription, DNA damage and viral replication. Myosins can act as molecular transporters and anchors that rely on their actin binding and ATPase capabilities. Their roles within the DNA damage response can varies from a transcriptional response, moving chromatin and stabilizing chromosome contacts. This review aims to highlight their key roles in the DNA damage response and how they impact nuclear organization and transcription.

Keywords; actin, damage, DNA, myosin, nucleus

A light way for nuclear cell biologists

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The nucleus is a very complex organelle present in eukaryotic cells. Having the crucial task to safeguard, organize and manage the genetic information, it must tightly control its molecular constituents, its shape and its internal architecture at any given time. Despite our vast knowledge of nuclear cell biology, much is yet to be unravelled. For instance, only recently we came to appreciate the existence of a dynamic nuclear cytoskeleton made of actin filaments that regulates processes such as gene expression, DNA repair and nuclear expansion. This suggests further exciting discoveries ahead of us. Modern cell biologists embrace a new methodology relying on precise perturbations of cellular processes that require a reversible, highly spatially confinable, rapid, inexpensive and tunEable external stimulus: light. In this review, we discuss how optogenetics, the state-of-the-art technology that uses genetically encoded light-sensitive proteins to steer biological processes, can be adopted to specifically investigate nuclear cell biology.

Keywords; dynamics, nucleus, optogenetics, photosensors, protein engineering

JB Special Issue—Regular Papers

Measuring nuclear calcium and actin assembly in living cells

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Nuclear calcium signalling has emerged as a critical mechanism regulating processes like chromatin organization and gene expression. Recently, we have shown that nuclear calcium elevation triggers rapid and transient actin filament assembly inside the nucleus. Here, we constructed and employed a nuclear-specific calcium sensor based upon the new generation of genetically encoded probes jGCaMP7f. By fusing a nuclear localization signal to jGCaMP7f, we achieved highly efficient nuclear-specific targeting. Comparing the jGCaMP7f-NLS probe with the previous GCaMP6f-NLS calcium sensor showed clearly that jGCaMP7f-NLS is more sensitive and reverses significantly quicker thereby reflecting rapid nuclear calcium transients in a closely physiological manner. We further confirm that nuclear calcium transients precede nuclear actin polymerization by several seconds. Our data show that calcium-triggered nuclear actin assembly in fibroblasts is independent of the actin nucleating Arp2/3 complex. Together, jGCaMP7f-NLS represents an easy to use, reliable and highly sensitive nuclear calcium sensor that allows to tightly interrogate real-time, spatiotemporal calcium signalling and calcium-elicited effects in the nucleus of living cells.

Keywords; calcium sensor, GCaMP, nuclear actin, nuclear calcium, nucleoskeleton

Modulating dynamics and function of nuclear actin with synthetic bicyclic peptides

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Actin exists in monomeric globular (G-) and polymerized filamentous (F-) forms and the dynamics of its polymerization/depolymerization are tightly regulated in both the cytoplasm and the nucleus. Various essential functions of nuclear actin have been identified including regulation of gene expression and involvement in the repair of DNA double-strand breaks (DSB). Small G-actin-binding molecules affect F-actin formation and can be utilized for analysis and manipulation of actin in living cells. However, these G-actin-binding molecules are obtained by extraction from natural sources or through complex chemical synthesis procedures, and therefore, the generation of their derivatives for analytical tools is underdeveloped. In addition, their effects on nuclear actin cannot be separately evaluated from those on cytoplasmic actin. Previously, we have generated

synthetic bicyclic peptides, consisting of two macrocyclic rings, which bind to G-actin but not to F-actin. Here, we describe the introduction of these bicyclic peptides into living cells. Furthermore, by conjugation to a nuclear localization signal (NLS), the bicyclic peptides accumulated in the nucleus. The NLS-bicyclic peptides repress the formation of nuclear F-actin, and impair transcriptional regulation and DSB repair. These observations highlight a potential role for NLS-linked bicyclic peptides in the manipulation of dynamics and functions of nuclear actin.

Keywords; actin-binding molecule, actin polymerization, bicyclic peptide, gene expression, nuclear actin

Visualization of endogenous nuclear F-actin in mouse embryos reveals abnormal actin assembly after somatic cell nuclear transfer

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Actin in the nucleus, referred to as nuclear actin, is involved in a variety of nuclear events. Nuclear actin is present as a globular (G-actin) and filamentous form (F-actin), and dynamic assembly/disassembly of nuclear actin profoundly affects nuclear functions. However, it is still challenging to observe endogenous nuclear F-actin. Here, we present a condition to visualize endogenous nuclear F-actin of mouse zygotes using different fixation methods. Zygotes fixed with paraformaldehyde and treated with fluorescently conjugated phalloidin show both short and long actin filaments in their pronuclei. Short nuclear actin filaments are characteristic of phalloidin staining, rather than the consequence of severing actin filaments by the fixation process, since long nuclear actin filaments probed with the nuclear actin chromobody are not disassembled into short filaments after fixation with paraformaldehyde. Furthermore, we find that nuclear actin assembly is impaired after somatic cell nuclear transfer (SCNT), suggesting abnormal nucleoskeleton structures in SCNT embryos. Taken together, our presented method for visualizing nuclear F-actin with phalloidin can be used to observe the states of nuclear actin assembly, and revealed improper reprogramming of actin nucleoskeleton structures in cloned mouse embryos.

Keywords; mouse, nuclear actin, phalloidin, somatic cell nuclear transfer, zygote

Lamin B receptor-mediated chromatin tethering to the nuclear envelope is detrimental to the *Xenopus* blastula

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In the nucleus of eukaryotic cells, chromatin is tethered to the nuclear envelope (NE), wherein inner nuclear membrane proteins (INMPs) play major roles. However, in *Xenopus* blastula, chromatin tethering to the NE depends on nuclear filamentous actin that develops in a blastula-specific manner. To investigate whether chromatin tethering operates in the blastula through INMPs, we experimentally introduced INMPs into *Xenopus* egg extracts that recapitulate nuclear formation in fertilized eggs. When expressed in extracts in which polymerization of actin is inhibited, only lamin B receptor (LBR), among the five INMPs tested, tethered chromatin to the NE, depending on its N2 and N3 domains responsible for chromatin-protein binding. N2–3-deleted LBR did not tether chromatin, although it was localized in the nuclei. We subsequently found that the LBR level was very low in the *Xenopus* blastula but was elevated after the blastula stage. When the LBR level was precociously elevated in the blastula by injecting LBR mRNA, it induced alterations in nuclear lamina architecture and nuclear morphology and caused DNA damage and abnormal mitotic spindles, depending on the N2–3 domains. These results suggest that LBR-mediated chromatin tethering is circumvented in the *Xenopus* blastula, as it is detrimental to embryonic development.

Keywords; blastula, chromatin tethering, inner nuclear membrane protein, lamin B receptor, *Xenopus*

BIOCHEMISTRY

Biochemistry General

Upregulation of circ_0000142 promotes multiple myeloma progression by adsorbing miR-610 and upregulating AKT3 expression

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Circular RNAs (circRNAs) play an important regulatory role in a variety of malignancies. Nevertheless, the role of circ_0000142 in multiple myeloma (MM) and its regulatory mechanism remains largely unknown. Real-time polymerase chain reaction was employed to detect the expressions of circ_0000142 and miR-610 in MM tissues and cell lines. The expression of AKT3 and apoptosis-related proteins (Bcl-2, Bax) in MM cells was detected by western blot. The correlation between the expression level of circ_0000142 and the clinicopathological parameters of MM patients was analysed. Cell proliferation, apoptosis, migration and invasion were monitored by Cell Counting Kit 8 assay, flow cytometry analysis and Transwell assay, respectively. The dual-luciferase reporter gene assay and RNA immunoprecipitation assay were employed to verify the targeting relationship between circ_0000142 and miR-610. In this study, it was demonstrated that, circ_0000142 was highly expressed in MM patients, and its high expression level was significantly associated with

increased International Staging System and Durie–Salmon stage. Overexpression of circ_0000142 enhanced MM cell proliferation, migration, invasion and suppressed cell apoptosis, while knocking down circ_0000142 had the opposite effects. Mechanistically, circ_0000142 functioned as a competitive endogenous RNA, directly targeting miR-610 and positively regulating AKT3 expression. In brief, circ_0000142 enhances the proliferation and metastasis of MM cells by modulating the miR-610/AKT3 axis.
Keywords; AKT3, ceRNA, circ_0000142, miR-610, MM

pH-mediated control of anti-aggregation activities of cyanobacterial and *E. coli* chaperonin GroELs

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In contrast to *Escherichia coli*, cyanobacteria have multiple GroELs, the bacterial homologues of chaperonin/Hsp60. We have shown that cyanobacterial GroELs are mutually distinct and different from *E. coli* GroEL with which the paradigm for chaperonin structure/function has been established. However, little is known about regulation of cyanobacterial GroELs. This study investigated effect of pH (varied from 7.0 to 8.5) on chaperone activity of GroEL1 and GroEL2 from the cyanobacterium *Synechococcus elongatus* PCC7942 and *E. coli* GroEL. GroEL1 and GroEL2 showed pH dependency in suppression of aggregation of heat-denatured malate dehydrogenase, lactate dehydrogenase and citrate synthase. They exhibited higher anti-aggregation activity at more alkaline pHs. *Escherichia coli* GroEL showed a similar pH-dependence in suppressing aggregation of heat-denatured lactate dehydrogenase. No pH dependence was observed in all the GroELs when urea-denatured lactate dehydrogenase was used for anti-aggregation assay, suggesting that the pH-dependence is related to some denatured structures. There was no significant influence of pH on the chaperone activity of all the GroELs to promote refolding of heat-denatured malate dehydrogenase. It is known that pH in cyanobacterial cytoplasm increases by one pH unit following a shift from darkness to light, suggesting that the pH-change modulates chaperone activity of cyanobacterial GroEL1 and GroEL2.
Keywords; chaperones, cyanobacteria, folding, GroEL, light reactions, photosynthesis

Double prenylation of SNARE protein Ykt6 is required for lysosomal hydrolase traffi cking

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Ykt6 is an evolutionarily conserved SNARE protein regulating Golgi membrane fusion and other diverse membrane trafficking pathways. Unlike most SNARE proteins, Ykt6 lacks a transmembrane domain but instead has a tandem cysteine motif at the C-terminus. Recently, we have demonstrated that Ykt6 undergoes double prenylation at the C-terminal two cysteines first by farnesyltransferase and then by a newly identified protein prenyltransferase named geranylgeranyltransferase type-III (GGTase-III). GGTase-III consists of a novel α subunit prenyltransferase alpha subunit repeat containing 1 (PTAR1) and the β subunit of Rab geranylgeranyltransferase. PTAR1 knockout (KO) cells, where Ykt6 is singly prenylated with a farnesyl moiety, exhibit structural and functional abnormalities in the Golgi apparatus with delayed intra-Golgi trafficking and impaired protein glycosylation. It remains unclear whether the second prenylation of Ykt6 is required for proper trafficking of lysosomal hydrolases from Golgi to lysosomes. Here, we show that lysosomal hydrolases, cathepsin D and β-hexosaminidase, were missorted at the *trans*-Golgi network and secreted into the extracellular space in PTAR1 KO cells. Moreover, maturation of these hydrolases was disturbed. LC3B, an autophagy marker, was accumulated in PTAR1 KO cells, suggesting defects in cellular degradation pathways. Thus, doubly prenylated Ykt6, but not singly prenylated Ykt6, is critical for the efficient sorting and trafficking of acid hydrolases to lysosomes.
Keywords; Golgi apparatus, lysosomal hydrolase, protein prenylation, SNARE, Ykt6

Protein Interaction and Recognition

Disparity of selenourea and selenocystine on methaemoglobinemia in non-diabetics and diabetics

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Organoselenium drugs like selenourea (SeU) and selenocystine (SeC) are found to exhibit several medicinal properties and have reported roles in the field of cancer prevention. However, studies related to their interactions with the major erythroid protein, haemoglobin (HbA) are still in dearth despite being of prime importance. In view of this, it was considered essential to investigate the interaction of these two anticancer drugs with Hb. Both the drugs showed significant changes in absorption spectra of Hb at wavelength of maximum absorption (λ_{max}) 630 nm. SeU itself had no effect on the absorbance value at 630 nm with respect to time even with 400 μM concentration. However, it was rapidly converted to nanoselenium in presence of nitrite and there was an increase in the absorbance rate at 630 nm from $3.39 \times 10^{-3}\,min^{-1}$ (without nitrite) to $8.94 \times 10^{-3}\,min^{-1}$ in pres-

ence of nitrite (200 μM) owing to the generation of reactive oxygen species in the medium. Although the generation and increase in peak intensity at 630 nm in Hb generally indicates the formation and rise in the levels of methaemoglobin (metHb), nanoselenium was observed to follow a different path. Instead of causing oxidation of Fe^{2+} to Fe^{3+} responsible for metHb formation, nanoselenium was found to interact with the protein part, thereby causing changes in its secondary structure which is reflected in the increasing absorbance at 630 nm. SeC, however, showed a different effect. It was shown to act as a novel agent to reduce nitrite-induced metHb formation in a dose-dependent manner. The efficiency of SeC was again found to be less in diabetic blood samples as compared to the non-diabetic ones. For similar ratio of metHb to SeC (1 : 8), % reduction of metHb was found to be 27.46 ± 0.82 and 16.1 ± 2.4 for non-diabetic and diabetic samples, respectively, with a two tailed *P*-value much <0.05 which implies that the data are highly significant.

Keywords; diabetic blood sample, methaemoglobin, reactive oxygen species, selenocystine, selenourea

Biochemical Pharmacology

Comparison of ameliorative effects of Taraxacum syriacum and N-acetylcysteine against acetaminophen-induced oxidative stress in rat liver and kidney

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Taraxacum syriacum (TS) with natural antioxidant and pharmacological activities may be considered for treatment of oxidative stress induced by acetaminophen (APAP). The aim of this study was to evaluate the ameliorative effects of the ethanol extract of TS root against hepatorenal toxicity induced by APAP in comparison to N-acetylcysteine (NAC) as a standard drug. Thirty male Wistar rats were randomly divided into five groups. Control group; APAP (1 g/kg) group; APAP–NAC (160 mg/kg) group and APAP-TS100 and APAP-TS200 groups: APAP plus 100 and 200 mg/kg of TS extract, respectively. After 7 days treatment, serum and liver and kidney tissues were prepared and evaluated. TS extract ameliorated the increased lipid peroxidation level and decreased antioxidant enzymes activities and glutathione level in liver and kidney of APAP-treated rats. Moreover, treatment with the TS extract caused significant reduction in the histopathological damages and high levels of serum biochemical markers of hepatic and renal functions after APAP treatment. This study suggests that the extract of TS roots has dose-dependent ameliorative effect against APAP-induced oxidative damage in liver and kidney due to its free radical scavenging and antioxidant properties. The overall efficacy of the extract at 200 mg/kg dose is comparable with NAC.

Keywords; acetaminophen, biochemical parameters, oxidative stress, rat, Taraxacum syriacum

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ダイジェスト

JB Commentary

The apelin/APJ system in the regulation of vascular tone: friend or foe?

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The apelin (APJ) receptor was originally cloned as a gene encoding a putative G protein-coupled receptor related to angiotensin receptor type I. To date, two endogenous peptide ligands for APJ have been identified: apelin and elabela/Toddler. The apelin/APJ system regulates blood pressure and vascular tone. The endothelial and smooth muscle apelin/APJ systems exert opposite actions in the regulation of vascular tone. Binding of apelin to endothelial APJ promotes the release of vasodilators, such as nitric oxide and prostacyclin, leading to vasodilation. Alternatively, binding of apelin to smooth muscle APJ induces vasoconstriction, although the molecular mechanisms of the apelin-induced vasoconstriction are poorly understood. Recently, a critical role for interaction of APJ with α1-adrenergic receptor in the apelin-induced vasoconstriction was reported. The action of apelin on vascular tone may depend upon blood vessel type or pathological condition. Although the apelin/APJ system could serve as a potential therapeutic target for hypertension and cardiovascular disease, the role of this system in various cell types appears to be complicated.

Keyword; apelin, APJ, blood pressure, G protein-coupled, vascular smooth muscle

JB Reviews

***Escherichia coli* amino acid auxotrophic expression host strains for investigating protein structure–function relationships**

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A set of C43(DE3) and BL21(DE3) *Escherichia coli* host strains that are auxotrophic for various amino acids is briefly reviewed. These strains require the addition of a defined set of one or more amino acids in the growth medium, and have been specifically designed for overproduction of membrane or water-soluble proteins selectively labelled with stable isotopes, such as ^{2}H, ^{13}C and ^{15}N. The strains described here are available for use and have been deposited into public strain banks. Although they cannot fully eliminate the possibility of isotope dilution and mixing, metabolic scrambling of the different amino acid types can be minimized through a careful consideration of the bacterial metabolic pathways. The use of a suitable auxotrophic expression host strain with an appropriately isotopically labelled growth medium ensures high levels of isotope labelling efficiency as well as selectivity for providing deeper insight into protein structure–function relationships.
Keyword; amino acid, auxotroph, Escherichia coli, isotope labelling, protein structure–function

Molecular functions of ASK family in diseases caused by stress-induced inflammation and apoptosis

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VCells are constantly exposed to various types of stress, and disruption of the proper response leads to a variety of diseases. Among them, inflammation and apoptosis are important examples of critical responses and should be tightly regulated, as inappropriate control of these responses is detrimental to the organism. In several disease states, these responses are abnormally regulated, with adverse effects. Apoptosis signal-regulating kinase (ASK) family members are stress-responsive kinases that regulate inflammation and apoptosis after a variety of stimuli, such as oxidative stress and endoplasmic reticulum stress. In this review, we summarize recent reports on the ASK family in terms of their involvement in inflammatory diseases, focussing on upstream stimuli that regulate ASK family members.
Keyword; ASK family, ER stress, inflammation, MAPK cascade, oxidative stress

Molecular and functional diversity of the oxytocinase subfamily of M1 aminopeptidases

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The placental leucine aminopeptidase/insulin-regulated aminopeptidase, endoplasmic reticulum aminopeptidase 1 and endoplasmic reticulum aminopeptidase 2 are part of a distinct subfamily of M1 aminopeptidases termed the 'oxytocinase subfamily'. The subfamily members show molecular diversity due to differential usage of translation initiation sites, alternative splicing and multiple single nucleotide polymorphisms. It is becoming evident that, depending on their intracellular or extracellular location, members of the oxytocinase subfamily play important roles in the maintenance of homeostasis, including the regulation of blood pressure, maintenance of normal pregnancy, retention of memory and trimming of antigenic peptides presented to major histocompatibility complex class I molecules, by acting as either aminopeptidases or binding partners of specific functional proteins in the cells. Based on their molecular diversity and moonlighting protein-like properties, it is conceivable that the subfamily members exert pleiotropic effects during evolution, to become important players in the regulation of homeostasis.
Keyword; endoplasmic reticulum aminopeptidase, insulin-regulated aminopeptidase, M1 aminopeptidase, oxytocinase subfamily, placental leucine aminopeptidase

BIOCHEMISTRY

Biochemistry General

Biochemical characterization of four splice variants of mouse Ca^{2+}/calmodulin-dependent protein kinase Iδ

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Ca^{2+}/calmodulin (CaM)-dependent protein kinase Iδ (CaMKIδ) is a Ser/Thr kinase that plays pivotal roles in Ca^{2+} signalling. CaMKIδ is activated by Ca^{2+}/CaM-binding and phosphorylation at Thr^{180} by CaMK kinase (CaMKK). In this study, we characterized four splice variants of mouse CaMKIδ (mCaMKIδs: a, b, c and d) found by *in silico* analysis. Recombinant mCaMKIδs expressed in *Escherichia coli* were phosphorylated by CaMKK; however, only mCaMKIδ-a and c showed protein kinase activities towards myelin basic protein *in vitro*, with mCaMKIδ-b and mCaMKIδ-d being inactive. Although mCaMKIδ-a and mCaMKIδ-c underwent autophosphorylation *in vitro*, only mCaMKI δ-c underwent autophosphorylation in 293T cells. Site-directed

mutagenesis showed that the autophosphorylation site is Ser^{349}, which is found in the C-terminal region of only variants c and b (Ser^{324}). Furthermore, phosphorylation of these sites (Ser^{324} and Ser^{349}) in mCaMKIδ-b and c was more efficiently catalyzed by cAMP-dependent protein kinase *in vitro* and *in cellulo* as compared to the autophosphorylation of mCaMKIδ-c. Thus, variants of mCaMKIδ possess distinct properties in terms of kinase activities, autophosphorylation and phosphorylation by another kinase, suggesting that they play physiologically different roles in murine cells.

Keyword; Camk1d, Ca^{2+}-signalling, characterization, protein kinase A, splice isoforms

Protein Structure

Glycosylation decreases aggregation and immunogenicity of adalimumab Fab secreted from Pichia pastoris

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Glycoengineering of therapeutic proteins has been applied to improve the clinical efficacy of several therapeutics. Here, we examined the effect of glycosylation on the properties of the Fab of the therapeutic antibody, adalimumab. An *N*-glycosylation site was introduced at position 178 of the H chain constant region of adalimumab Fab through site-directed mutagenesis (H:L178N Fab), and the H:L178N Fab was produced in *Pichia pastoris*. Expressed mutant Fab contained long and short glycan chains (L-glyco Fab and S-glyco Fab, respectively). Under the condition of aggregation of Fab upon pH shift-induced stress, both of L-glyco Fab and S-glyco Fab were less prone to aggregation, with L-glyco Fab suppressing aggregation more effectively than the S-glyco Fab. Moreover, the comparison of the antigenicity of glycosylated and wild-type Fabs in mice revealed that glycosylation resulted in the suppression of antigenicity. Analysis of the pharmacokinetic behaviour of the Fab, L-glyco Fab and S-glyco Fab indicated that the half-lives of glycosylated Fabs in the rats were shorter than that of wild-type Fab, with L-glyco Fab having a shorter half-life than S-glyco Fab. Thus, we demonstrated that the glycan chain influences Fab aggregation and immunogenicity, and glycosylation reduces the elimination half-life *in vivo*.

Keyword; aggregation, Fab, glycoengineering, immunogenicity, pharmacokinetic

Protein Interaction and Recognition

Analysis of binding residues in monoclonal antibody with high affinity for the head domain of the rat P2X4 receptor

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P2X4 receptor is known to be involved in neuropathic pain. In order to detect the expression of P2X4 receptor on microglia at the time of onset of neuropathic pain, one approach consists on the preparation of the monoclonal antibodies with both selective binding and high affinity. We have recently established a monoclonal antibody (named 12–10H) which had high affinity to rat P2X4 receptor expressed in 1321N1 cells. The dissociation constants of the complex between the monoclonal antibodies obtained so far and the head domain (HD) in the rat P2X4 receptor were in the nanomolar range. To improve the affinity by rational mutations, we need to know the precious location of the binding site in these monoclonal antibodies. Here, we have analysed and identified the binding residues in the monoclonal antibody (12–10H) with high affinity for the HD of the rat P2X4 receptor by site-directed mutagenesis.

Keyword; monoclonal antibody, neuropathic pain, P2X4 receptor, protein–protein interaction, refolding

Enzymology

Genetic analysis of tellurate reduction reveals the selenate/tellurate reductase genes ynfEF and the transcriptional regulation of moeA by NsrR in *Escherichia coli*

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Several bacteria can reduce tellurate into the less toxic elemental tellurium, but the genes responsible for this process have not yet been identified. In this study, we screened the Keio collection of single-gene knockouts of *Escherichia coli* responsible for decreased tellurate reduction and found that deletions of 29 genes, including those for molybdenum cofactor (Moco) biosynthesis, iron–sulphur biosynthesis, and the twin-arginine translocation pathway resulted in decreased tellurate reduction. Among the

gene knockouts, deletions of *nsrR*, *moeA*, *yjbB, ynbA, ydaS* and *yidH* affected tellurate reduction more severely than those of other genes. Based on our findings, we determined that the *ynfEF* genes, which code for the components of the selenate reductase YnfEFGH, are responsible for tellurate reduction. Assays of several molybdoenzymes in the knockouts suggested that *nsrR*, *yjbB*, *ynbA*, *ydaS* and *yidH* are essential for the activities of molybdoenzymes in *E. coli.* Furthermore, we found that the nitric oxide sensor NsrR positively regulated the transcription of the Moco biosynthesis gene *moeA*. These findings provided new insights into the complexity and regulation of Moco biosynthesis in *E. coli*.

Keyword; *Escherichia coli*, molybdenum cofactor, NsrR, tellurate reductase, transcriptional regulator

MOLECULAR BIOLOGY

Molecular Biology General

Identification of novel heat shock-induced long non-coding RNA in human cells

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The heat-shock response is a crucial system for survival of organisms under heat stress. During heat-shock stress, gene expression is globally suppressed, but expression of some genes, such as chaperone genes, is selectively promoted. These selectively activated genes have critical roles in the heat-shock response, so it is necessary to discover heat-inducible genes to reveal the overall heat-shock response picture. The expression profiling of heat-inducible protein-coding genes has been well-studied, but that of non-coding genes remains unclear in mammalian systems. Here, we used RNA-seq analysis of heat shock-treated A549 cells to identify seven novel long non-coding RNAs that responded to heat shock. We focussed on CTD-2377D24.6 RNA, which is most significantly induced by heat shock, and found that the promoter region of CTD-2377D24.6 contains the binding site for transcription factor HSF1 (heat shock factor 1), which plays a central role in the heat-shock response. We confirmed that HSF1 knockdown cancelled the induction of CTD-2377D24.6 RNA upon heat shock. These results suggest that CTD-2377D24.6 RNA is a novel heat shock-inducible transcript that is transcribed by HSF1.

Keyword; heat-shock stress, HSF1, long non-coding RNA, RNA-seq analysis, stress response

Identification and analysis of short open reading frames (sORFs) in the initially annotated noncoding RNA LINC00493 from human cells

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Whole transcriptome analyses have revealed that mammalian genomes are massively transcribed, resulting in the production of huge numbers of transcripts with unknown functions (TUFs). Previous research has categorized most TUFs as noncoding RNAs (ncRNAs) because most previously studied TUFs do not encode open reading frames (ORFs) with biologically significant lengths [>100 amino acids (AAs)]. Recent studies, however, have reported that several transcripts harbouring small ORFs that encode peptides shorter than 100 AAs are translated and play important biological functions. Here, we examined the translational capacity of transcripts annotated as ncRNAs in human cells, and identified several hundreds of ribosome-associated transcripts previously annotated as ncRNAs. Ribosome footprinting and polysome profiling analyses revealed that 61 of them are potentially translatable. Among them, 45 were nonnonsense-mediated mRNA decay targets, suggesting that they are productive mRNAs. We confirmed the translation of one ncRNA, LINC00493, by luciferase reporter assaying and western blotting of a FLAG-tagged LINC00493 peptide. While proteomic analysis revealed that the LINC00493 peptide interacts with many mitochondrial proteins, immunofluorescence assays showed that its peptide is mitochondrially localized. Our findings indicate that some transcripts annotated as ncRNAs encode peptides and that unannotated peptides may perform important roles in cells.

Keyword; ncRNAs, ribosomes, short peptides, sORFs, TUFs

Gene Expression

Involvement of GcvB small RNA in intrinsic resistance to multiple aminoglycoside antibiotics in *Escherichia coli*

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Deleting the gene for small RNA GcvB in *Escherichia coli* was found to increase the sensitivity to several aminoglycoside antibiotics, such as neomycin, streptomycin, kanamycin, kasugamycin and spectinomycin, at low concentrations. GcvB, conserved

in gram-negative enteric bacteria, is known to negatively control the expression of many genes for amino acid incorporation systems, especially the periplasmic ABC-transporter proteins. Deletions of several amino acid transporter genes in Δ*gcvB* cells decreased the antibiotic sensitivity to the wild-type level, suggesting that those genes are involved in uptake of aminoglycosides into the cell. Since GcvB is constitutively synthesized in growing cells, repressing synthesis of amino acid transporters, it contributes to the intrinsic resistance to several aminoglycoside antibiotics.

Keyword; amino acid transporter, aminoglycoside antibiotics, *E.coli*, GcvB, intrinsic resistance

CELL

Biomembranes

Functional complementation of V-ATPase a subunit isoforms in osteoclasts

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In osteoclasts, the *a*3 isoform of the proton-pumping V-ATPase plays essential roles in anterograde trafficking of secretory lysosomes and extracellular acidification required for bone resorption. This study examined functional complementation of the *a* isoforms by exogenously expressing the *a*1, *a*2 and *a*3 isoforms in *a*3-knockout (KO) osteoclasts. The expression levels of *a*1 and *a*2 in *a*3KO osteoclasts were similar, but lower than that of *a*3. *a*1 significantly localized to lysosomes, whereas *a*2 slightly did. On the other hand, *a*2 interacted with Rab7, a regulator of secretory lysosome trafficking in osteoclasts, more efficiently than *a*1. *a*1 partly complemented the functions of *a*3 in secretory lysosome trafficking and calcium phosphate resorption, while *a*2 partly complemented the former but not the latter function.

Keyword; a subunit isoform, osteoclast, Rab7, secretory lysosome, V-ATPase

Cell Death

CircRNA_0092516 regulates chondrocyte proliferation and apoptosis in osteoarthritis through the miR-337-3p/PTEN axis

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The dysregulation of circular RNAs (circRNAs) has been identified in various human diseases. Here, we probed into the potential mechanism of circRNA_0092516 in osteoarthritis (OA). The expression of circRNA_0092516 was tested by quantitative real-time PCR. MTT, flow cytometry and western blot were applied to confirm the functions of circRNA_0092516 *in vitro*. Besides, RNA pull-down and dual-luciferase reporter gene experiments were applied to probe into the mechanism. circRNA_0092516 was raised in the tissues of OA patients and chondrocytes stimulated by IL-1*β*. The potential mechanism analysis expounded that circRNA_0092516 bound to miR-337-3p, and the interference with circRNA_0092516 boosted chondrocyte proliferation and restrained cell apoptosis through the miR-337-3p/phosphatase and tensin homolog (PTEN) axis, thereby improving OA. *In-vivo* experiments expounded that circRNA_0092516 regulated cartilage production through miR-337-3p. Overall, our data expounded that the interference with circRNA_0092516 boosted chondrocyte proliferation and restrained cell apoptosis through the miR-337-3p/PTEN axis, eventually slowed down the progress of OA.

Keyword; chondrocyte, circRNA_0092516, miR-337-3p, PTEN, osteoarthritis