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ダイジェスト

JB REVIEW

DDX5: a versatile RNA helicase in cancer and cell fate determination—linking c-Myc and JAK2 V617F signalling to emerging therapeutics

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Traditionally regarded as a DEAD-box RNA helicase involved in RNA metabolism, DDX5 (also known as p68) has emerged as a signal-responsive regulator that integrates RNA processing with transcriptional control, thereby influencing cell fate determination and tumourigenesis. Beyond its canonical roles in pre-mRNA splicing, ribosome biogenesis, microRNA maturation and R-loop resolution, DDX5 also functions in a helicase-independent manner as a transcriptional co-activator for diverse transcription factors and signalling pathways. Recent studies have revealed a dichotomy in which the RNA helicase activity and scaffolding/co-activator functions of DDX5 are selectively deployed in a context-dependent manner. In cancer, DDX5 enhances the transcriptional activity and transforming capacity of the proto-oncogene c-Myc and is itself transcriptionally induced by c-Myc, forming a positive feedback loop that sustains oncogenic transcriptional programs. DDX5 is also indispensable for transformation driven by the constitutively active JAK2 V617F mutant, a major driver of myeloproliferative neoplasms, where it promotes malignant signalling largely independently of its RNA helicase activity. In contrast, during adipocyte differentiation, the helicase function of DDX5 is essential for glucocorticoid receptor transcriptional programs. Importantly, recent studies have identified the small-molecule compound FL118 as a potent DDX5 degrader, providing proof of concept that targeting DDX5 protein stability represents a promising therapeutic strategy.

Keywords: adipocyte differentiation, c-Myc signalling, DDX5, helicase-independent function, JAK–STAT pathway

Regulatory dynamics of monosomes and polysomes in cellular adaptation

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Cytosolic 80S ribosomes have long been considered a uniform translation apparatus, with the only distinction being that they exist as either monosomes or polysomes. However, emerging evidence has revealed that monosomes and polysomes exhibit distinct translational profiles, contributing to selective protein synthesis and localized messenger RNA (mRNA) translation in specific subcellular compartments in mammals. When mammalian cells encounter environmental or metabolic stress, global gene expression is dynamically reprogrammed, accompanied by a marked shift in the monosome-to-polysome ratio, suggesting context-dependent roles for monosome- and polysome-mediated mRNA translation in cellular adaptation. In parallel, accumulating evidence indicates that distinct types of eukaryotic non-translating monosomes are formed under various biological conditions. Moreover, monosomes participate in the first round of mRNA translation on newly synthesized mRNAs, a process coupled with several early events such as nonsense-mediated mRNA decay and messenger ribonucleoprotein remodelling. In this review, we summarize current advances in understanding the roles of monosomes as crucial regulatory layers that shape the proteome across diverse physiological conditions in mammals.

Keywords: monosome, mRNA, translation, polysome, ribosome

RAPID COMMUNICATION

Phosphatidylinositol 3,4-bisphosphate emerges as a lipid mediator linking oxidative stress to DNA damage

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Reactive oxygen species function as physiological signalling molecules but, when excessive, cause oxidative stress that damages DNA. Using quantitative phosphoinositide profiling by regioisomer-resolving mass spectrometry, we identified phosphatidylinositol 3,4-bisphosphate [PI(3,4)P_2] as a phosphoinositide class robustly induced by hydrogen peroxide. Biochemical and genetic analyses demonstrated that stress-induced PI(3,4)P_2 production was predominantly dependent on the phosphoinositide 5-phosphatase Src homology 2 domain–containing inositol 5-phosphatase 2 (SHIP2). SHIP2 deficiency markedly suppressed PI(3,4)P_2 accumulation and reduced oxidative stress–

induced DNA damage. Oxidative stress increased PI(3,4)P_2 levels in the nuclear fraction, and intracellular delivery of PI(3,4)P_2 was sufficient to induce DNA damage independently of oxidative stress. These findings identify PI(3,4)P_2 as a lipid mediator linking oxidative stress to genome instability and uncover a SHIP2-dependent remodelling pathway that connects redox perturbation to DNA damage responses.

Keywords: DNA damage, oxidative stress, phosphoinositide, PI(3,4)P2, SHIP2

REGULAR PAPERS

BIOCHEMISTRY

Analytical Biochemistry

Quantification of L- and D-enantiomers of proline, trans-4-hydroxyproline and cis-4-hydroxyproline in natural samples using differential labelling with 12C- and 13C6-dabsyl chloride, chiral separation and liquid chromatography-mass spectrometry

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L-Proline (L-Pro) and *trans*-4-hydroxy-L-proline (t-4-HPL) are major components of collagen and plant cell wall proteins. During their breakdown, free L-Pro and t-4-HPL are produced abundantly and play unique biological roles through complex metabolisms including isomerization. Therefore, it is essential to quantify all the six stereoisomers of proline and 4-hydroxyproline. Here, analytes were first labelled with 12C-dabsyl group, which has strong red colour and stability under light. Secondly, each of the 13C6-dabsyl stereoisomers were added to 12C-dabsylated samples as internal standards before cleanup. Finally, the molar ratio of 12C- and 13C6-dabsyl analytes was determined as the intensity ratio of doublet molecular ions with *m*/*z* difference of six in mass spectra, which were obtained by subjecting the cleaned sample to capillary chromatography-mass spectrometry. The total amounts of proline, t-4-HP and *cis*-4-hydroxyproline were determined using the standard addition method. Linear calibration curves ($R2 > 0.99$) were obtained in the range of 10–1000 pmol. To determine the molar ratio of D- and L-enantiomers, enantiopure dabsyl analyte fraction was obtained using reverse-phase and chiral chromatography. Mouse serum contained 0.20 μmol/L *trans*-4-hydroxy-D-proline. *Achatina fulica* haemolymph contained 0.070 μmol/L D-proline. The *Santalum album* wood powder contained all the 6 stereoisomers and unexpectedly the D-enantiomer was dominant for *trans*-4-hydroxyproline.

Keywords: chiral separation, isotope dilution, matrix effects, secondary amino acids, standard addition

MOLECULAR BIOLOGY

Molecular Biology General

Recurrent mutations in protein tyrosine phosphatase receptor-type kappa in depressed-type colorectal carcinomas

Gene Expression

Severe hyperosmotic stress causes dysregulation of replication-dependent histone mRNA expression and induces DNA replication stress

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The expression of replication-dependent (RD) histones is tightly regulated by several pathways, including transcription and mRNA processing. However, the detailed mechanisms underlying the regulation of specific RD histone genes remain to be elucidated. Here, we demonstrated the differential responses of RD histone genes to severe hyperosmotic stress. Our data revealed an increased polyadenylation of the mRNA of some RD histones, although the RD histone mRNAs are known as to lack a poly(A) tail. Other RD histone genes showed elevated expression in total mRNA level. The nuclear bodies that mediate RD histone mRNA regulation, Cajal bodies, were disorganized after severe hyperosmotic stress. Depletion of the small nuclear RNA U7 (Rnu-7), which is involved in transcriptional suppression and processing of RD histone mRNAs, results in an increase in total mRNA levels with an identical specificity to hyperosmotic stress, but not polyadenylation. These findings demonstrated that RD histone genes were differentially regulated under normal conditions and in response to cellular stress.

Keywords: Cajal body, DNA replication stress, histone, hyperosmotic stress, Rnu-7

CELL

Tumor and Immunology

Dynamic changes of the sympathetic nervous system in the bone marrow in myeloid leukaemia

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Chronic myeloid leukaemia (CML) is a hematologic malignancy originating from hematopoietic stem cells with the fusion oncogene BCR–ABL1 on the Philadelphia chromosome, which drives the abnormal proliferation of leukemic blast cells within the bone marrow microenvironment. While previous research has primarily focused on the hematopoietic compartment, the functional contribution of the bone marrow microenvironment to the CML pathology remains understudied. We investigated the changes in the peripheral nervous system in the bone marrow with myeloid leukaemia via immunofluorescence staining of tyrosine hydroxylase (TH) and calcitonin gene-related peptide (CGRP) antibodies in mouse with NUP98–HOXA9– and BCR–ABL1—expressing myeloid leukaemia. We found that the TH-positive fibres were significantly reduced, while no overt changes were observed in CGRP-positive nerves in the bone marrow. The reduction in TH-positive nerve cells was also evident in the spleen. Human patient gene expression data suggested that the levels of sympathetic nerve receptor expression change during the blastic transformation of human CML. Our findings indicate that the sympathetic nervous system regulates the pathogenesis of myeloid leukaemia and could play a crucial role in the disease progression of myeloid leukaemia.
Keywords: bone marrow, microenvironment, myeloid leukaemia, peripheral nervous system, sympathetic nervous system

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JB REVIEW

A two-tiered epigenetic mechanism ensures imprint stability after fertilization: insights from the Igf2/H19 locus

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Genomic imprinting is a crucial epigenetic mechanism directing parent-of-origin-specific gene expression in mammals, and it is regulated by allele-specific DNA methylation at imprinting control regions (ICRs). In this canonical imprinting mechanism, maintenance factors preserve germline-derived DNA methylation at ICRs during genome-wide demethylation after fertilization. However, our studies of the *Igf2/H19* locus reveal that allele-specific *de novo* DNA methylation after fertilization, termed 'post-fertilization imprinted methylation', also contributes to imprint maintenance. In this process, epigenetic imprints other than DNA methylation are thought to guide the recognition of parental alleles by *de novo* methyltransferases in the next generation, acting in concert with maintenance factors to ensure stable DNA methylation at canonical imprinted loci. Recently identified non-canonical imprinting, in contrast, relies on non-DNA methylation marks that are converted into DNA methylation only after implantation. Despite differences in the timing of this conversion, post-fertilization imprinted methylation may share similarities with non-canonical imprinting. Collectively, these findings suggest that the robustness of canonical imprinting is supported by a two-tiered regulatory system.
Keywords: DNA methylation, genome imprinting, imprinting control region, transgenerational epigenetic inheritance, transgenic mice

Time to rethink circadian rhythms beyond the transcriptome

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The circadian clock generates ~24-hour rhythms in physiology by coordinating gene expression programs, but temporal mRNA profiles often fail to predict their protein function rhythms. This gap reflects regulatory layers beyond transcription, including rhythmic translation, protein stability, subcellular localization and post-translational modifications that collectively determine the circadian rhythms of protein abundance and activity. Here, we summarize evidence supporting a shift from RNA-level descriptions to protein-level frameworks and readouts of the circadian rhythms. Here, we highlight three topics: (i) protein abundance rhythms as informative but incomplete readouts, (ii) widespread circadian control of nuclear localization and phosphorylation that can occur without changes in total protein levels, and (iii) multi-tissue proteomic comparisons that reveal how circadian rhythms are organized differently across tissues. We then discuss how recent data-independent acquisition-based, high-throughput mass spectrometry accelerates cross-study reuse and hypothesis generation, as illustrated by a mouse circadian proteome atlas and an interactive portal enabled by Orbitrap Astral mass spectrometer. Together, these advances motivate 'functional chronobiology', linking proteome dynamics to mechanism and disease-relevant physiology.
Keywords: circadian rhythm, proteomics, mass spectrometry, post-translational modification, protein localization

Zinc-mediated structural and functional regulation of ERp44 and ERGIC-53 in protein quality control

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Zinc ions (Zn^{2+}) are essential trace metal ions in the human body. Intracellular Zn^{2+} levels are tightly regulated by two metal transporter families: ZIPs, which mediate Zn^{2+} influx into the cytosol, and ZnTs, which export Zn^{2+} from the cytosol to the extracellular space or sequester it into the cellular organelles. Within cells, Zn^{2+} plays multiple roles, acting as a catalytic cofactor for numerous enzymes, stabilizing protein structures, and functioning as a second messenger in signal transduction. In addition, Zn^{2+} is involved in the transient regulation of enzymatic activities. Here, we review recent findings that reveal novel roles of Zn^{2+} in the structural and functional regulations of the molecular chaperone ERp44 and the cargo receptor ERGIC-53, both of which operate for protein quality control in the early secretory pathway.

Keywords: cargo transport, ERGIC-53, ERp44; MCFD2, protein quality control, zinc

REGULAR PAPERS

BIOCHEMISTRY

Biochemistry General

Dynamics of CDKL5 phosphorylation and its regulatory mechanisms in cultured cells

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Cyclin-dependent kinase-like 5 (CDKL5) is a serine/threonine protein kinase highly expressed in the brain, and mutations in its gene cause CDKL5 deficiency disorder (CDD). Although reports on CDKL5 substrate proteins are increasing, the phosphorylation-dependent regulation of CDKL5 function remains poorly understood. Therefore, in this study, we investigated the phosphorylation states of CDKL5 and explored the kinases and phosphatases involved in its regulation. The C-terminal region of exogenous CDKL5 was highly phosphorylated in Neuro2a neuroblastoma cells; endogenous CDKL5 in P19 embryonic carcinoma (P19EC) cells was also highly phosphorylated, mainly in the cytoplasm. CDKL5 underwent gradual dephosphorylation during aggregate formation in P19EC cells, and this process was suppressed by retinoic acid (RA), an inducer of neuronal differentiation. Okadaic acid treatment indicated that protein phosphatase 2A (PP2A) partially mediates CDKL5 dephosphorylation during aggregate formation. Kinase inhibitor screening and kinase assay *in vitro* demonstrated that multiple protein kinase C (PKC) isoforms contribute to CDKL5 phosphorylation. These findings suggest that CDKL5 phosphorylation is dynamically regulated by a balance between PKC and PP2A activities, and that RA signalling modulates this process during neuronal differentiation. Such phosphorylation mechanisms of CDKL5 may underlie the pathogenesis of CDD and help identify potential therapeutic targets.

Keywords: cyclin-dependent kinase-like 5 (CDKL5), Phos-tag SDS-PAGE, protein kinase C (PKC), protein phosphatase 2A (PP2A), protein phosphorylation

BIOTECHNOLOGY

Gene and Protein Engineering

Ribosome display-derived CD207-binding peptides enhance humoral responses via Langerhans cell-targeted antigen delivery

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Langerhans cells (LCs) are specialized antigen-presenting cells residing in the epidermis. LCs express the C-type lectin receptor CD207, which mediates antigen uptake and internalization. Because epidermal LCs encounter antigens at the skin surface and regulate subsequent immune responses, CD207 provides a rational molecular target for skin-based antigen delivery strategies. In this study, we performed ribosome display using a structurally constrained cyclic peptide library to identify mCD207-binding peptides. After three rounds of bio-panning, three candidate peptide-EGFP fusion constructs exhibited micromolar affinity for recombinant mCD207, with the strongest binder showing a K_D of ~0.3 μM. Peptide 2 and peptide 3 are selectively bound to mCD207-expressing mammalian cells and primary mouse LCs. Fusion of peptide 3 to a model antigen enhanced antigen delivery to LCs and increased antigen-specific serum IgG levels following intradermal immunization in mice. These findings identify ribosome display-derived mCD207-targeting peptides as novel molecular tools for selective antigen delivery to epidermal LCs.

Keywords: antigen-presenting cells, CD207, directed evolution, peptides, ribosome display

Biochemical Pharmacology

Cannabidiolic acid causes a defect in tail retraction of migrating MDA-MB-231 cells: possible involvements of Rho-associated protein kinases inhibition and accumulation of vinculin at the rear of migrating cells

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We previously reported that cannabidiolic acid (CBDA), a major cannabinoid constituent of the fiber-type cannabis plant, abrogates the migration of highly aggressive human breast cancer MDA-MB-231 cells and activates the small GTPase RhoA by inhibiting protein kinase A. However, the mechanism(s) mediating RhoA signaling, which decreases cell migration, have not yet been comprehensively elucidated. RhoA is an upstream mediator of Rho-associated kinases (ROCKs), diaphanous-related formins (DIAPHs), the RhoA-ROCK pathway (tail retraction) and the RhoA-DIAPH pathway (lamellipodia formation). Herein, we identified CBDA as an inhibitor of ROCKs (at approximately 25μM), which markedly elongated the cell body of MDA-MB-231 cells, similar to Y-27632, an established ROCK inhibitor. CBDA stimulated lamellipodia formation at the leading edge, whereas NSC23766 (an established Rac1 inhibitor) completely blocked this elongated morphology. Biochemical analyses, including time-lapse imaging and confocal laser scanning microscopy, revealed that, compared to Y-27632, CBDA can induce impaired tail retraction coupled with unidirectional elongation of the cell body, upregulate the mRNA expression of *DIAPH*s and accumulate vinculin, an adhesion protein, at the trailing edge without affecting its expression. These results indicate the potential of CBDA as a new candidate for the synthesis of ROCK inhibitors, which can evoke the directed elongation of MDA-MB-231 cells.

Keywords: cannabidiolic acid, directed elongation, MDA-MB-231 cells, Rho-associated kinases, vinculin

MOLECULAR BIOLOGY

Gene Expression

Individual short-chain fatty acids diff erentially reprogram transcriptional states in colorectal cancer cells

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Short-chain fatty acids (SCFAs) produced by the gut microbiota contribute to intestinal function, immune responses and host metabolism, yet how individual SCFAs shape intracellular gene expression states remains incompletely defined. Here, we used intestinal epithelial-derived colorectal cancer cells to characterize early responses to sodium butyrate, propionate, valproate, acetate and 3-hydroxybutyrate using RNA sequencing. RNA-seq showed that SCFA treatment altered transcriptional states, with distinct effects among SCFA species. Functional analyses suggested a tendency toward suppression of stimulus-responsive signalling pathways across colorectal cancer cell lines, together with downregulation of pathways involved in RNA processing and post-transcriptional regulation. In addition, intron retention increased after SCFA treatment. Although the magnitude depended on treatment conditions, reproducible intron retention patterns were detected in specific cell lines, consistent with potential alterations in RNA maturation processes. Analyses of H3K27me3 and RT-qPCR further suggested that some SCFA-induced expression changes were transient, whereas others were partially sustained in association with altered epigenetic profiles. Collectively, these datasets provide a foundation for understanding how microbiota-derived SCFAs influence cellular states in colorectal cancer cell lines through transcriptional changes and increased intron retention.

Keywords: colorectal cancer cells, epigenome, HDAC inhibitor, intron retention, short-chain fatty acids

CELL

Cell General

Endothelial RhoA is essential for embryonic and postnatal vascular development by regulating KLF2–Notch1 signaling

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The small GTPase RhoA has diverse cellular functions, including in actin fiber reorganization. However, its role in angiogenesis remains elusive. To reveal this, we generated mice with endothelium-specific constitutive or tamoxifen-inducible conditional knockout (cKO) of RhoA. Both constitutive and inducible RhoA cKO mice all died by embryonic day 11.5. They displayed severe abnormalities in cephalic plexus vasculature and intersomitic vessels. Inducible RhoA cKO mice also had abnormal lymphatic vessels at mid-stage embryogenesis and showed significant reductions in radial outgrowth and density of vascular plexus in the retina on postnatal day 10. Blood flow recovery and vascular repair in ischemic hindlimbs were also impaired in adult inducible RhoA cKO mice. The expression of a stalk cell marker Notch1 was reduced, but that of a tip cell marker Dll4 was unchanged in the branched vessels of RhoA cKO embryos. By luciferase analysis and a database search, KLF2 was identified as a negative regulator of Notch1 expression by binding to the *NOTCH1* gene promoter. KLF2 was phosphorylated by the RhoA–ROCK pathway, which inhibited KLF2 nuclear translocation, resulting in increases in Notch1 transcription and expression. Endothelial RhoA supports endothelial differentiation through KLF2–Notch1 signaling and promotes embryonic and postnatal vascular development.

Keywords: angiogenesis, KLF2, neovascularization, Notch1, small GTPase

Receptors and Signal Transduction

Adhesion G protein-coupled receptor F5 mediates shear stress mechanotransduction in glomerular endothelial cells

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Adhesion G protein-coupled receptor F5 (ADGRF5) is widely expressed and is involved in various physiological and pathophysiological functions. ADGRF5 has been demonstrated to be expressed in glomerular endothelial cells and to maintain the integrity of the glomerular filtration barrier by regulating the expression of type IV collagens COL4A3 and COL4A4. However, the mechanisms underlying its activation remain unclear. Herein, we showed that ADGRF5 overexpression enhances ERK1/2 phosphorylation induced by laminar flow shear stress in human embryonic kidney PEAKrapid cells. Inhibitor and knockdown experiments revealed that the ADGRF5-mediated shear stress response proceeds via the $G\alpha_{q/11}$–protein kinase C–RAF1–MEK1/2–ERK1/2 cascade. Mutagenesis analyses showed that shear flow-mediated activation of ADGRF5 requires the tethered agonist (*Stachel*) sequence and the C-terminal helix 8. Consistent with the overexpression analyses, endogenous ADGRF5 mediated shear stress-induced ERK1/2 signalling in human primary renal glomerular endothelial cells. Moreover, ADGRF5 knockdown attenuated shear flow-induced upregulation of the immediate early response genes and the type IV collagen genes *COL4A3* and *COL4A4*. These results suggest that ADGRF5 mediates shear stress mechanotransduction in glomerular endothelial cells and regulates endothelial cell responses, with potential implications for glomerular filtration barrier homeostasis.

Keywords: collagen, ERK1/2, glomerular endothelial cell, GPCR, shear stress