

JB REVIEW

RNA structural biology of neurodegeneration: phase separation, phase transition and proteinopathy

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Neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease, are becoming increasingly prevalent in super-ageing societies. However, the molecular mechanisms by which prion-like proteins undergo aberrant phase transition, aggregation and propagation during neurodegeneration remain incompletely understood. Although these proteins are capable of undergoing liquid-liquid phase separation (LLPS), followed by a sol-gel transition *in vitro*, the factors that govern their pathological phase transition *in vivo* remain largely elusive. Notably, many prion-like proteins also possess RNA-binding properties, and accumulating evidence indicates that RNA plays pivotal roles in regulating both LLPS and the subsequent transition to more solid-like states. In this review, we summarize recent advances in the RNA structural biology of neurodegeneration, with particular emphasis on RNA G-quadruplexes (rG4s) as pathological determinants and discuss emerging mechanisms by which RNA structures promote the phase transition and proteinopathy of prion-like proteins.

Keywords: liquid-liquid phase separation, neurodegenerative disorders, prion-like proteins, RNA G-quadruplex, RNA structural biology

BNIP3/NIX-dependent mitophagy: molecular mechanisms and physiological roles

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Mitochondria are essential for cellular metabolism and homeostasis, and their quality and quantity must therefore be tightly controlled. Mitophagy, a selective form of autophagy targeting mitochondria, contributes to this control by eliminating dam-

aged or superfluous mitochondria. Among the known mitophagy pathways, BNIP3/NIX-dependent mitophagy has emerged as a key mechanism, particularly under hypoxic and metabolic stress. Recent studies have provided important insights into how BNIP3 and NIX are transcriptionally induced, post-translationally regulated and functionally coupled to the core autophagy machinery. These studies have also clarified their roles in isolation membrane tethering, membrane elongation and mitophagosome formation. Beyond its molecular basis, accumulating evidence indicates that BNIP3/NIX-dependent mitophagy contributes to mitochondrial homeostasis, redox balance and cellular stress adaptation. This review summarizes recent progress in understanding the molecular mechanisms and physiological significance of BNIP3/NIX-dependent mitophagy.

Keywords: BNIP3, mitochondrial quality control, mitophagy, NIX/BNIP3L, receptor-mediated mitophagy

REGULAR PAPERS

BIOCHEMISTRY

Protein Structure

Structure and biochemical analyses suggest that PrkA/YeaG protein of *Thermus thermophilus* functions as a putative molecular chaperone

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Protein phosphorylation, a key post-translational modification, is mediated by various protein kinases. The bacterial PrkA/YeaG is recognized as an atypical protein kinase, but its activity remains elusive. This study investigated the structural and functional characteristics of a PrkA/YeaG homologue, TpkB, from *Thermus thermophilus* HB8. Using cryo-electron microscopy, the structures of TpkB in apo and AMPPNP-bound forms were determined, revealing a hexameric ring architecture characteristic of AAA⁺ superfamily proteins. The TpkB protomer is comprised

of an N-terminal domain, an ATPase domain and an LID domain. The ATPase domain contains conserved sequence motifs associated with ATPase activity, whereas the other domains present a novel fold. TpkB exhibits structural similarity to MoxR family proteins, which possess chaperone-like functions in conjunction with von Willebrand factor A (vWA) domain proteins. Structural and gene neighbourhood analyses suggested a functional link between PrkA/YeaG proteins and vWA domain proteins. Biochemical analyses demonstrated that TpkB exhibited ATPase activity and chaperone-like activity, but lacked detectable protein kinase activity. These findings establish TpkB as a novel member of the AAA⁺ superfamily with potential chaperone functions, providing new insights into the PrkA/YeaG family.

Keywords: AAA⁺ superfamily, cryo-electron microscopy, protein kinase, tertiary structure, zinc-binding protein

Protein Interaction and Recognition

Involvement of the OB-fold and the C-terminal acidic tip of SSB in its interaction with RecO from *Thermus thermophilus* HB8

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In bacterial homologous recombination, the single-stranded DNA (ssDNA)-binding protein (SSB) coats exposed ssDNA to protect it but simultaneously inhibits RecA filament nucleation. RecO, a recombination mediator, overcomes this inhibition by interacting with SSB and displacing it from ssDNA. This SSB-RecO interaction has long been considered to rely primarily on the conserved C-terminal acidic tip (C-tip) of SSB. Here, using domain-truncated SSB variants from *Thermus thermophilus* HB8, we show that RecO directly binds both the SSB oligonucleotide/oligosaccharide-binding fold (OB-fold) and the C-tip. These two contacts make distinct contributions to RecO's mediator functions. Arg127 in RecO contributes to recognition of the SSB C-tip and to double-stranded DNA (dsDNA) binding, the latter of which is suppressed by the SSB C-tip. In RecO-mediated ssDNA annealing, the SSB C-tip primarily modulates SSB dynamics on ssDNA rather than directly activating RecO. Moreover, stable assembly of the SSB-RecO-RecR ternary complex requires the SSB C-tip. Our findings support a mechanism in which the SSB C-tip modulates RecO function and governs SSB behaviour on ssDNA, whereas the OB-fold provides additional contacts whose functional contributions remain to be elucidated.

Keywords: homologous recombination, protein-protein interaction, recombination mediator, single-stranded DNA-binding protein, *Thermus thermophilus* HB8

Nucleic Acid and Peptide Biochemistry

NMR analysis of interaction between RNA structure elements and small molecules: evaluation of RNA libraries and confirmation by two more molecules

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RNA-targeted small molecule drug discovery is widely recognized as an important modality. We have demonstrated that NMR spectroscopy is useful for screening small molecules that bind to specific RNA structures. In the previous work, we analysed the changes in the imino proton signals of 47 RNAs with different structures; it was clearly indicated upon addition of three well-known small molecules, risdiplam, NCD, and CPFX and found several combinations of small molecules and RNA structures that showed specific changes. In this study, the structural characteristics of the model RNAs were further analysed by measuring the *T_m* values. Then, to demonstrate the importance of the NMR-based method, we applied the method to two additional small molecules. In addition to the imino proton signals, the H5-H6 signals were also used to detect the interaction between small molecules and single-stranded regions of RNAs. As a result, two additional examples of specific RNA-small molecule interactions were identified.

Keywords: NMR, RNA-targeted drug discovery

Metabolism and Bioenergetics

Substrate specificity of an archaeal thioesterase from *Thermococcus kodakarensis*

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Thioesterases catalyse the hydrolysis of thioester bonds and are responsible for reactions such as the release of fatty acids from

acyl-acyl carrier protein in fatty acid biosynthesis. Although enzymes from bacteria and eukaryotes have been studied, identification and characterization of thioesterases from archaea have not been reported. Here, we searched the genome of the hyperthermophilic archaeon *Thermococcus kodakarensis* for candidate genes that might encode a thioesterase. Among these, the TK1652 protein contained a HotDog fold and shared ~20% identity with TE8 and TE13 family thioesterases. Active site residues proposed for TE8 and TE13 enzymes were partially conserved in the TK1652 protein. The recombinant TK1652 protein produced in *Escherichia coli* was purified to apparent homogeneity. Although TE8 and TE13 family enzymes assemble as tetramers, the TK1652 protein formed a homodimer. The protein displayed thioesterase activity toward acetyl-CoA, and to a much lower extent, propionyl-CoA. The TK1652 protein did not recognize phenylacetyl-CoA, *n*-decanoyl-CoA, lauroyl-CoA and myristoyl-CoA, substrates utilized by TE8/TE13 family thioesterases. Differences in active site residues, quaternary structure and substrate specificity suggested that the TK1652 protein represents a new family of thioesterases. Acetyl-CoA thioesterase activity was detected in cell-free extracts of *T. kodakarensis*, and the metabolic roles of this activity are discussed.

Keywords: archaea, enzyme, hyperthermophile, metabolism < nucleoside/nucleotides, thioesterase

BIOTECHNOLOGY

Synthetic Peptides and Oligonucleotides

Kinetic and thermodynamic properties of phosphorothioate and phosphorodiamidate morpholino oligonucleotides binding to target RNA

Immunological Engineering

Development and validation of a novel monocyte activation test with secreted luciferase-expressing cells

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Pyrogen testing is essential for ensuring pharmaceutical product safety; however, current methods have limitations. The Bacterial Endotoxins Test cannot detect non-endotoxin pyrogens, whereas

the Rabbit pyrogen test was phased out for animal welfare reasons. Monocyte activation tests (MAT), using human whole blood samples or peripheral blood mononuclear cells, are effective but suffer from donor variability, biosafety concerns and instability. We aimed to develop a sensitive, rapid and simple alternative pyrogen test by devising a luciferase-based MAT using the Mono-Mac-6 (MM6) cell line. MM6 cells showed higher cytokine responses to endotoxin than THP-1 cells and exhibited comparable or greater sensitivity than human peripheral blood. Introducing an NF- κ B-driven NanoLuc[®] reporter enabled endotoxin detection within 3 h; however, rapid degradation of intracellular luciferase was noted. To overcome this degradation issue, we generated MM6 cells expressing secreted NanoLuc (secNluc), allowing for stable detection between 3 and 12 h after stimulation. The MM6/NF κ B-secNluc cells helped detect various pyrogens, including Pam3CSK4, peptidoglycan, polyinosinic-polycytidylic acid sodium salt, lipopolysaccharide, flagellin, macrophage-activating lipopeptide-2 and resiquimod. Adding recombinant human lipopolysaccharide-binding protein enabled establishing a serum-free MAT system, eliminating serum-derived endotoxin contamination. This reporter-based MAT provides a robust platform for rapid endotoxin and NEP detection, offering an improved alternative to conventional MATs.

Keywords: Luciferase, MAT, monocyte activation test, NF- κ B, pyrogen test

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ダイジェスト

JB Review

Nutrigenomic regulation of one-carbon metabolism and the circadian clock in health and disease

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Methylation of DNA, histones and RNA is central to the regulation of circadian rhythms, yet the biochemical origin of the methyl groups driving these modifications has received comparatively little attention in circadian biology. This review explores the bidirectional crosstalk between the methyl cycle and the mammalian circadian clock. We describe how S-adenosylmethionine-dependent epigenetic and epitranscriptomic modifications constitute essential layers of circadian gene regulation, and how the clock orchestrates the rhythmic expression of one-carbon

metabolism enzymes and oscillations in S-adenosylmethionine availability. The direct interaction between the *S-adenosylhomocysteine hydrolase* and the core clock component BMAL1 at circadian gene promoters emerges as a molecular nexus linking methyl group supply to clock-driven transcription. We further discuss how the methyl cycle occupies a privileged position within the circadian entrainment hierarchy, acting as both a target of nutritional zeitgebers in peripheral tissues and a potential source of metabolic feedback to the central pacemaker, and how dietary perturbation of the methyl cycle disrupts circadian rhythms. Finally, we discuss how this crosstalk is implicated in metabolic liver disease, cancer, neurological disorders and aging. Together, these findings position the circadian clock as a sensitive read-out of nutritional methyl metabolic status, with broad implications for chronobiology and nutrigenomics.

Keywords: circadian clock, epigenetics, epitranscriptomics, one-carbon metabolism, *S-adenosylmethionine*

Chromatin dynamics and cellular energy homeostasis

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Metabolism is a complex consortium of chemical reactions, consuming and releasing energy. In addition, cellular respiration is a pivotal catabolic process of nutrients using oxygen to generate ATP. Dynamics of chromatin structure are fundamental to genomic events in all cellular processes. Therefore, chromatin consumes robust cellular energy formed in metabolism. To regulate cellular processes, chromatin functions and metabolism need to be tightly coordinated in response to environmental changes including nutrition availability. Indeed, molecular oxygen directly impacts DNA and chromatin modification and influences cell fate. Herein, we review fundamentals of chromatin dynamics, nucleosome modifications and roles of chromatin dynamics in spatiotemporal regulation of cellular energy homeostasis.

Keywords: chromatin, histone modification, metabolism, nucleosome, transcription

REGULAR PAPERS

BIOCHEMISTRY

Protein Interaction and Recognition

Amino acid residues responsible for the different pH dependency of photosynthetic type and non-photosynthetic-type ferredoxins in the electron transfer reaction with ferredoxin-NADP⁺ reductase from maize leaves

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In higher plants, ferredoxin (Fd) is present as distinct isoproteins of photosynthetic type (LFd) and non-photosynthetic type (RFd), which exhibit differential function despite their similarity in the 3D structures. We previously showed that pH-dependency of electron transfer activity with ferredoxin-NADP⁺ reductase (FNR) was opposite between LFd and RFd, which was explained by the opposite pH-dependent profile of K_m for the two Fds, and that the differences of C-terminal residues and 78th residue between the two Fds were partly responsible for the different pH dependency. In this study, we further investigated the determinants responsible for the different pH dependency between the LFd and RFd. Site-directed mutants of Fd, substituted at the residues on the interface with FNR, were prepared. Kinetic analyses using the single site-directed mutants and their combined, multiple mutants showed that combination of the substitutions of Fd residues at 61, 63, 78th and C-terminal region located at the interface with FNR conferred opposite pH-dependency, which indicated that these residues are the enough determinant for their opposite pH dependency of LFd and RFd in the electron transfer reaction with FNR.

Keywords: electron transfer complex, ferredoxin, ferredoxin-NADP⁺ reductase, pH-dependency, protein-protein interaction

Multiple binding modes of the tyrosine kinase inhibitor dovitinib to fibroblast growth factor receptor 1 characterized by surface plasmon resonance, ¹⁹F-NMR and molecular dynamics simulations

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Fibroblast growth factor receptor 1 (FGFR1) is a receptor-type tyrosine kinase involved in various human cancers, making it an attractive therapeutic target. To elucidate the complex binding dynamics between FGFR1 and its highly selective inhibitor, dovitinib, we conducted a detailed investigation using a combination of surface plasmon resonance (SPR), ¹⁹F-NMR spectroscopy and molecular dynamics (MD) simulations. Kinetic analysis using SPR suggested that the interaction between FGFR1 and dovitinib is better described by a two-state binding model, implying the potential formation of an initial transient intermediate prior to the formation of a stable complex. We further characterized this interaction using ¹⁹F-NMR by leveraging the intrinsic fluorine atoms of dovitinib. When dovitinib was pres-

ent in excess relative to FGFR1, the ^{19}F -NMR signal exhibited broadening compared to that in the free state, indicating altered dovitinib environments and additional FGFR1-associated states. Furthermore, MD simulations introducing an additional dovitinib molecule to the FGFR1–dovitinib complex indicated the potential for weak and transient interactions in the preferred interaction regions on the FGFR1 surface. These findings highlight the multifaceted nature of kinase–inhibitor interactions and provide valuable biophysical insights that may contribute to future drug discovery efforts targeting receptor tyrosine kinases.

Keywords: differential scanning fluorimetry, molecular dynamics, NMR, surface plasmon resonance, tyrosine kinase inhibitor

Regulatory mechanism of heme-regulated inhibitor through autophosphorylation-driven activation and heme-induced deactivation

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Heme-regulated inhibitor (HRI) is a key modulator of hemoglobin synthesis, exerting such effects by sensing intracellular heme levels. Under heme-deficient conditions, HRI dissociates from heme, becomes activated and phosphorylates the translation initiation factor eIF2 α . However, the precise regulatory mechanisms governing HRI activation remain incompletely understood. In this study, we delineate part of the regulatory mechanism involving autophosphorylation-dependent activation and heme-mediated deactivation of HRI. HRI formed a dimer in solution through its N-terminal domain, irrespective of its phosphorylation state. However, a mutant with deletion of the N-terminal domain retained autophosphorylation activity, indicating that N-terminal domain-mediated dimerization is not essential for activation. Phosphorylated HRI formed a stable complex with eIF2 α , whereas the dephosphorylated form failed to bind, indicating that autophosphorylation is required for eIF2 α recognition. Biochemical analyses, together with modelling based on the predicted structure, revealed that the phosphate groups of Thr488 and Thr493 interact with adjacent basic residues. These interactions enhance eIF2 α recognition and phosphorylation.

Additionally, heme-induced deactivation selectively targeted the dephosphorylated kinase domain and suppressed autophosphorylation. These findings provide multiple mechanistic insights into regulation of the activity of HRI, highlighting distinct roles for autophosphorylation, structural elements and heme responsiveness in controlling its function.

Keywords: eIF2 α heme protein, HRI, ISR, translation

CELL

Receptors and Signal Transduction

TGF- β downregulates E-cadherin expression via a novel ZEB1-dependent mechanism

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Zinc finger E-box-binding homeobox 1 (ZEB1) is a core transcription factor regulating epithelial–mesenchymal transition (EMT) and its high expression is associated with malignant cancer phenotypes and poor prognosis. ZEB1 mediates the downregulation of E-cadherin (encoded by *CDH1*) as a hallmark of cells undergoing EMT. We previously identified two distinct modes of E-cadherin downregulation by ZEB1, namely basal repression and stimulus-dependent repression. The current study aimed to investigate the mechanisms responsible for stimulus-dependent repression induced by transforming growth factor- β (TGF- β) using *ZEB1*-knockout cells. Knockout of *ZEB1* resulted in upregulation of basal E-cadherin expression and attenuation of its downregulation following TGF- β treatment. TGF- β -induced E-cadherin downregulation required Smad3 signalling, but did not involve upregulation of ZEB1 expression, suggesting functional modulation of ZEB1. C-terminal binding proteins were required for basal repression but not for TGF- β -induced downregulation. Consistently, basal repression of *CDH1* by ZEB1, but not TGF- β -induced downregulation, was associated with histone deacetylation in the *CDH1* regulatory regions; however, both processes required histone deacetylase activity. Histone deacetylase may therefore play a role in regulating the expression or function of factor(s) required for TGF- β -induced *CDH1* downregulation. These results suggest that TGF- β downregulates E-cadherin expression via a novel ZEB1-dependent mechanism.

Keywords: E-cadherin, EMT, HDAC, TGF- β , ZEB1