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Light regulates nuclear detainment of intron-retained transcripts through COP1-spliceosome to modulate photomorphogenesis

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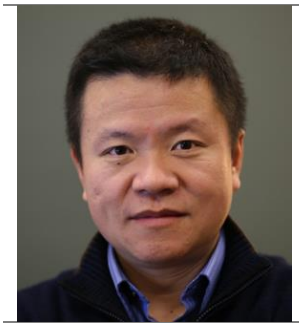
Abstract

Intron retention (IR) is the most common alternative splicing (AS) event in *Arabidopsis*. Increasing studies have demonstrated a major role of IR in gene expression regulation. The impacts of IR on plant growth and development, and response to environments remains under explored. Here, we found that IR functions directly in gene expression regulation on a genome-wide scale through detainment of the intron-retained transcripts (IRTs) in the nucleus. Through this mechanism, nuclear-retained IRTs can be kept away from translation. COP1-dependent light modulation of IRTs of light signaling genes, such as *PIF4*, *RVE1*, and *ABA3*, contribute to seedling morphological development in responding to changing light conditions. Further, light-induced IR changes are under the control of the spliceosome, and in part through COP1-dependent ubiquitination and degradation of DCS1, a plant-specific spliceosomal component. Our data suggests that light regulates the activity of the spliceosome and the consequent IRTs nucleus detainment to modulate photomorphogenesis through COP1.



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Converging Mechanisms of Cancer Mutations and A Molecular Glue Degrader

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Abstract

Molecular glue (MG) degraders represent an exciting new modality in drug discovery highlighted by their ability to degrade undruggable targets and their favorable pharmacological properties. Thus far, only a very small number of MG degraders have been serendipitously found, most of which act on CULLIN4 E3 ubiquitin ligases. The scarcity of these novel compounds limits our understanding of their activity requirement, E3 ligases preference, and functional versatility, thereby hindering the prospective discovery of new MG degraders. UM171 is a potent small molecule agonist of ex vivo human hematopoietic stem cell (HSC) self-renewal, a process that is tightly controlled by epigenetic regulation. By co-opting KBTBD4, a substrate receptor of the CULLIN3-RING E3 ubiquitin ligase complex, UM171 promotes the degradation of members of the CoREST transcriptional corepressor complex, thereby limiting HSC attrition. Interestingly, KBTBD4 also is recurrently mutated in medulloblastoma (MB), the most common embryonal brain tumor in children. These mutations impart gain-of-function to KBTBD4 to induce aberrant degradation of CoREST as well. In this study, we find that UM171 acts as a molecular glue degrader of CoREST by bridging its binding partner HDAC1 and the E3 ligase KBTBD4. Remarkably, the action of UM171 requires an endogenous metabolite, inositol hexakisphosphate, as a second molecular glue. We also discover that the cancer mutations of KBTBD4 structurally, functionally, and mechanistically mimic UM171. Our results reveal a converging mechanism between a molecular glue degrader and human disease mutations, which points to an exciting “genetics-first” functional genomics strategy for identifying glue-able E3-target pairs for rational discovery of new molecular glue degraders.

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Rewiring of Cooperative Stabilization Networks in PCI complexes

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Abstract

Protein complexes are fundamental to all cellular processes, so understanding their evolutionary history and assembly processes is important. Paralogous protein complex families arising from gene duplication and subsequent divergence are crucial to how protein complexes evolve. Nonetheless, whether paralogous complexes are assembled via similar pathways and how cross-complex interference is avoided remain unanswered questions. Subunits of protein complexes are often stabilized upon complex formation, whereas unincorporated subunits are degraded. How such cooperative stability influences protein complex assembly also remains unclear. Here, we demonstrate that subcomplexes driven by cooperative stabilization interactions serve as building blocks for protein complex assembly. We have further developed a protein stability-guided method to compare the assembly processes of paralogous PCI (Proteasome lid, CSN, eIF3) complexes *in cellulo*. Our findings support that oligomeric state and the structural organization of paralogous complexes can be maintained even if their assembly processes are rearranged. Our results indicate that divergent assembly processes by paralogous complexes not only enable the complexes to evolve new functions, but also reinforce their segregation by establishing incompatibility against deleterious hybrid assemblies.

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The COP9 signalosome: The regulator of CRLs

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Abstract

The COP9 signalosome (CSN) controls cullin-RING-ubiquitin ligases (CRLs). The CSN is not only involved in the remodeling of CRLs, but it also regulates ubiquitylation of the CRL substrates. In contrast to its paralog complexes the 26S proteasome LID and the eukaryotic translation initiation factor 3 (eIF3), the CSN consists of variants which are distinguished by paralog subunits like CSN7A and CSN7B. The variants are called CSN^{CSN7A} and CSN^{CSN7B} and regulate the CRLs CRL3 and CRL4, respectively. Our data show, that the two complexes, CSN^{CSN7A}-CRL3 and CSN^{CSN7B}-CRL4, possess different functions during adipogenesis. The knockout of CSN7B leads to uncontrolled p27 degradation by CRL4A preventing adipogenesis. The loss of CSN^{CSN7A}-CRL3 is associated with the disappearance of lipid droplets (LDs) and block of adipogenesis. Electron microscopy shows that RAB18 recruits CSN^{CSN7A}-CRL3 to LDs where it fulfills specific functions. The stability of CSN^{CSN7A}-CRL3 and CSN^{CSN7B}-CRL4 complexes are assured by the C-termini of CSN7A and of CSN7B. Transfection of FLAG-$CSN7A^{1-200}$ or FLAG-$CSN7B^{1-200}$ into LiSa-2 cells and integration into CSN complexes leads to inhibition of adipogenesis.

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Global remodeling of the proteome in terminal differentiation

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Abstract

Erythropoiesis exemplifies one of the major adaptations at the proteome level in nature, where progenitors with a complex proteome differentiate into erythrocytes, in which a remarkable 98% of soluble protein content is hemoglobin. The mechanisms that drive removal of myriad pre-existing components are for the most part unidentified. To study this process, we have generated murine mutations in components of the ubiquitin system (UPS) and assayed the erythroid remodeling process by quantitative global proteomics. The findings show how specific components of the UPS trigger the elimination of independent components of the cell on a vast scale, eliminating many of the basic functionalities of cell biology. The talk will focus on the elimination of ~100 RNA-regulatory proteins by the CTLH ubiquitin ligase. The turnover of >150 mRNA species is perturbed as a result. The capacity of CTLH to drive this ubiquitination program is dependent an erythroid-lineage-specific factor that is highly induced in the program, namely YPEL5. Reconstitution of the ubiquitination of RNA-regulatory proteins in reticulocyte lysate with recombinant YPEL5, and mutational analysis of YPEL5, support the hypothesis that YPEL5 is a specificity factor for CTLH. Interestingly, YPEL5 is closely related to cereblon, although CTLH is not in the cullin family. Our work indicates a new capacity of the UPS, to effect global and developmentally controlled proteomic remodeling.

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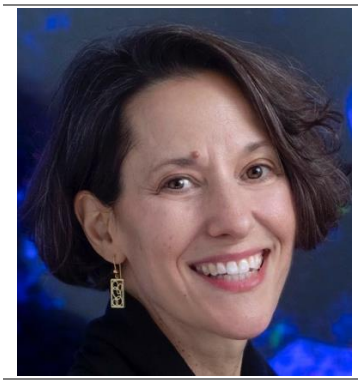

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Structural insights into proteasome assemblies

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Abstract

Members of our group have become interested in assemblies of a classic "Zome", the proteasome. We have been investigating structures ranging from biogenesis of the 20S core particle (collaborations with John Hanna and Wade Harper) to proteasome assemblies in cells including yeast (collaborations with Cordula Enenkel, Wade Harper, and Wolfgang Baumeister). In my talk, I will present our latest data in this field.

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Stress responding mechanisms of the 26S proteasome and their pathological implications

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Abstract

The 26S proteasome is a self-compartmentalized protease complex, one of whose crucial functions is protein quality control. Multiple layers of regulatory systems elaborately modulate proteasomal activity, i.e., hydrolysis of polyubiquitinated proteins. However, the mechanism of destruction of mammalian proteasomes is poorly understood. We found that inactive 26S proteasomes are concentrated into an insoluble aggresome. These proteasomes were colocalized with autophagic receptor SQSTM1/p62 in a large perinuclear inclusion body and were cleared through selective macroautophagy, linking aggresomal segregation to proteaphagic degradation. This pathway might be counterbalanced with recovery of proteasomal activity and critical for reducing cellular proteasomal stress. The amount and activity of proteasomes are also subject to structural alteration and transcriptional regulation. More specifically, glucose starvation uncouples 26S holoenzymes into 20S and 19S subcomplexes and immunoproteasome subunits are expressed in fibrotic kidney cell. These are cellular response mechanisms to adapt hostile environments for cell survival. Thus, 26S proteasomes are controlled by transcriptional and post-translational mechanisms, which is crucial for not only proteasome quality control and but also overall protein homeostasis.

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Separately prestored proteasome components to prevent polyspermy

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Abstract

An egg can only accommodate a single sperm to maintain the genome stability across generations. How sperm trigger polyspermy prevention during fertilization is still largely unknown. Here, we show that 20S proteasome components prestored in the sperm head which subsequently participates in polyspermy prevention. The depletion of 20S proteasome components, but not 19S components, from spermatids results in polyspermy in the zygotes; and the knockout of 19S components from oocytes also leads to polyspermy. We found that testis-specific 20S components were incorporated into oocyte-derived 19S proteasome components to promote the degradation of Fetuin B, which is ubiquitinated by MARCH3. After Fetuin B clearing, ZP2 can be cleaved, and the zona pellucida will be hardened, thus preventing other sperm enter into the egg. The newly assembled proteasome represents a novel strategy to quickly prevent polyspermy during fertilization in mammals.

Key words: Proteasome components, ubiquitination, oocytes, sperm, polyspermy

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AMP Synthesis Inhibition-Proteasome Promotes Proteostasis Mediated Longevity and Health Span across Species

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Abstract

Disruption of protein homeostasis is a fundamental process and driver of aging. Protein homeostasis is affected by the degradation efficiency of accumulated protein aggregates, but it is unclear whether and how protein homeostasis can be restored by drugs to extend the lifespan and healthy lifespan of eukaryotes. Here, we identified 5-iodotuberculin (5-ITu), an inhibitor of the AMP biosynthetic kinase ADK, that confers potent anti-aging potency on yeast cells, worms, mammalian cells, and mice. Using a yeast model overexpressing AD-derived toxic TDP43 aggregates, we screened and found that 5-Itu mitigated the toxicity of protein aggregates. We then confirmed the anti-aging, longevity, and healthy lifespan-promoting effects of 5-ITu in yeast cells, Caenorhabditis elegans, mammalian cells, and mice. Mechanistically, 5-ITu inhibits ADK, resulting in increased proteasome activity and improved protein degradation involving AMP-activated protein kinases (AMPKs). In addition, we show that restriction of AMP biosynthesis via glucose pathway, methionine pathway, or salvage adenine pathway confers anti-aging effects, respectively, implying that anti-aging therapies based on calorie restriction and nutrient restriction have a uniform mode of action. This study paves the way for the identification of new protein homeostasis maintenance interventions to improve longevity and healthy longevity.

Key words: AMP synthesis, Proteasome, Proteostasis, Longevity, 5-Iodotubercidin

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Proteasome Regulation in Health and Disease

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Abstract

The ubiquitin-proteasome system is responsible for degradation of the majority of cellular proteins in eukaryotes. Often dubbed as the cellular trashcan, the proteasome has been widely misconceived as being static, constitutively active and solely afloat in the cytosol. Our work has contributed to understanding how the assembly, activity and substrate selectivity of the proteasome can be spatiotemporally regulated by proteasome-interacting proteins and chemical modifications (esp. phosphorylation) in response to various signals, reshaping the local or global proteome. We also recently characterized a special pool of proteasomes that associate with cellular membranes via N-myristoylation, which play surprisingly important roles in embryonic development, tumorigenesis and viral replication. These findings call for rethinking current strategies for targeted protein degradation, and have fertilized an “old field” from which new research and therapeutic tools may sprout.

Key words: 26S proteasome, Phosphorylation, N-Myristoylation, Membrane proteins, DNA repair

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Deregulation of CSN6-SPOP-HMGCS1 axis promotes hepatocellular carcinoma progression

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Abstract

Mevalonate (MVA) pathway dysregulation in liver cancer remains not well-characterized. Here we show that roles of CSN6 in YAP activation/Cholesterol metabolism are involved in liver cancer. CSN6 is elevated and is a positive regulator of hydroxymethylglutaryl-CoA synthase 1 (HMGCS1) of MVA pathway to promote tumorigenesis in HCC. CSN6 antagonizes speckle-type POZ protein (SPOP) ubiquitin ligase to stabilize HMGCS1, thereby activating YAP1 to promote HCC growth. Mechanistically, CSN6-MDM2 axis can mediate SPOP ubiquitination. Significantly, combination of YAP signaling inhibitor verteporfin and HMGCS1 knockdown treatment is efficient in treating CSN6-high HCC. Harnessing CSN6-SPOP-HMGCS1 axis bear important prognostic and therapeutic implications for improving therapeutic efficacy of HCC.

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Control of ADP-heptose-induced NF-kB activity by CSN-associated USP48 in the *Helicobacter pylori* infected gastric mucosa

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Abstract

The COP9 signalosome (CSN) and CSN-associated deubiquitinylases (DUBs) control nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-kB) in the *Helicobacter pylori* infected gastric mucosa. The human microbial pathogen *H. pylori* is a risk factor for the development of gastric diseases including cancer. The *H. pylori* lipopolysaccharide metabolite ADP-D-glycero-b-D-manno-heptose (ADP-Hep) binds to the alpha kinase 1 (ALPK1) and triggers the cell signaling leading to activation of NF-kB. The CSN-associated DUB USP48 stabilizes NF-kB/RelA by deubiquitinylation and thereby promotes the transcriptional activity. We have investigated the interplay of CSN-associated DUBs in classical and alternative NF-kB signaling in the context of gastric pathology.

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Hectd3 inhibits tumor growth by reversing immunosuppressive protumoral properties of TAM

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Abstract

Tumor-associated macrophages (TAM) are a kind of normal cells abundant in tumor microenvironment, which can promote tumor growth, invasion and metastasis. TAM is a very attractive anticancer target due to its clear pro-tumor effect. TAM has strong plasticity and is obviously biased towards protumoral M2 polarization during tumor progression. Reversing TAM into cancer-suppressing M1 polarization is a potential cancer treatment strategy. IFNs-STAT1 signaling pathway plays an important role in M1 macrophage polarization. Herein, our study suggests that E3 ubiquitin ligase HECTD3 mediates polyubiquitination modification of STAT1 and maintain its protein stability, promotes M1 polarization of TAM, and inhibits tumor growth. In this study, we used a variety of mouse models to demonstrate that Hectd3 deficiecy in macrophage significantly promoted the growth of tumor. Hectd3$^{-/-}$ TAM and BMDM (Bone marrow derived macrophage) secreted less IFN-β, and the expression of characteristic genes of M1 macrophage decreased while that of M2 macrophage increased. Hectd3 inhibited E0771 and 4T1-Luc tumor growth dependent on CD8+ T cell immune response. Mechanically, we found that Hectd3 deletion decreased the protein level of STAT1 and the level of STAT1 activation in macrophage when cocultured with tumor cells. The DBD domain of STAT1 and the DOC domain of Hectd3 mediated protein-protein interaction. Further ubiquitin experiments showed that Hectd3 promoted the K33 chain polyubiquitination of STAT1. These results suggest that Hectd3 may be a key molecule that reverses the immunosuppressive protumoral properties of TAM, which provides a new potential target for tumor therapy.

Key Words: Tumor-associated macrophages (TAM), Hectd3, STAT1, polyubiquitination

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Functions of the COP9 signalosome (CSN) as a regulator of SCF $^{EBF-PIF3}$ in *Arabidopsis thaliana* and SCF $^{Grr-A}$ in *Beauveria bassiana*

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Abstract

Functions of the SKP1-CUL1-F box (SCF) ubiquitin E3 ligases are essential in plants. With more than 800 F-box protein genes, they participate in broad aspects of plant development. The F box proteins (FBPs) are substrate receptors that recruit substrates and assemble an active SCF complex, but the regulatory mechanism underlying the FBPs binding to CUL1 to activate the SCF cycle is not fully understood in plants. We show that Arabidopsis csn1-10 is defective in SCF^{EBF1}-mediated PIF3 degradation during de-etiolation, due to impaired association of EBF1 with CUL1 in csn1-10. EBF1 preferentially associates with un-neddylated CUL1 that is deficient in csn1-10 and the EBF1-CUL1 binding is rescued by the neddylation inhibitor MLN4924. Furthermore, we identify a subset of FBPs with impaired binding to CUL1 in csn1-10, indicating their assembly to form SCF complexes may depend on COP9 signalosome (CSN)-mediated deneddylation of CUL1. This study reports that a key role of CSN-mediated CULLIN deneddylation is to gate the binding of the FBP-substrate module to CUL1, thus initiating the SCF cycle of substrate ubiquitination.

Beauveria bassiana is a broad-spectrum and environmentally friendly fungal pesticide. *B. bassiana* contains a conserved COP9 signalosome complex (BbCSN) of 7 subunits. Our study show that BbCSN is necessary for the function and stability of GrrA in SCF ^{GrrA}-mediated growth and pathogenesis of the fungus

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Regulation of CRL neddylation dynamics upon osmotic stress

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Abstract

Cullin-RING ubiquitin ligases control the stability of regulators of essential cellular processes, including hormone signalling and abiotic stress response. CRL activity is switched on by the conjugation of the ubiquitin-like modifier NEDD8 to their cullin subunit (neddylation), which is catalysed by an enzymatic cascade which includes the NEDD8 E1 subunit AXR1, and switched off by NEDD8 deconjugation (deneddylation), which is catalysed by the COP9 signalosome (CSN). Both neddylation and deneddylation are critical for CRL function and plant viability, as mutations disrupting these processes result in developmental arrest. High saline concentration in soil is one of the major constraints in agriculture. Here, we show that high salinity induces a rapid accumulation of NEDD8-CUL1in Arabidopsis seedlings, and that mutations in cullin neddylation and deneddylation, which have opposite effects on NEDD8-CUL1 levels, exacerbate salt sensitivity in germinating seeds. This suggests that a reversible switch of CUL1 to NEDD8-CUL1 is crucial to ensure a prompt salt stress response. Interestingly, water deprivation similarly triggers NEDD8-CUL1 accumulation across different Arabidopsis ecotypes, indicating that this mechanism might be part of the plant response to osmotic stress. We are currently investigating the molecular basis of the salt-induced accumulation of NEDD8-CUL1. Understanding this process could unveil new strategies to enhance plant resilience in the face of environmental changes.

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This abstract reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

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The blue-light receptor CRY1 serves as a switch to balance photosynthesis and defense

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Abstract

Plants open their stomata for gas exchange and water transpiration in response to blue light, but open stomata are an opportunity for pathogen entry. Whether plants can sense danger when their stomata open in response to blue light and are prepared for pathogen defense remains unknown, and how blue-light cues are integrated into to balancing growth–defense trade-offs are poorly characterized. Here we show that the Arabidopsis photoreceptor CRYPTOCHROME 1 (CRY1) mediates pathogen-triggered stomata closure under blue light through a typical light-responsive protein LATE UPREGULATED IN RESPONSE TO *HYALOPERONOSPORA PARASITICA* (LURP1). LURP1 undergoes N-terminal palmitoylation in the presence of bacterial flagellin, prompting a change in its subcellular localization from the cytoplasm to plasma membrane where it enhances the immune-activation activity of the receptor FLAGELLIN SENSING 2 (FLS2). This work uncovers a blue-light-dependent regulation of stomatal defense and highlights a dual function of CRY1 in photosynthesis and immunity.

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Hormone-mediated disassembly and inactivation of a plant E3 ubiquitin ligase complex

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Abstract

Phytohormone abscisic acid (ABA) regulates key aspects of plant development such as seed germination, as well as responses to important environmental stresses, including drought, salinity and cold temperatures. The ABA signaling pathway is tightly controlled by the ubiquitin proteasome system. CULLIN4-RING E3 ubiquitin ligases use the substrate receptor module COP10-DDB1-DET1-DDA1 (CDDD) to target PYL8, one of the ABA receptors of the PYR/PYL/RCAR (pyrabactin resistance/pyrabactin resistance-like/regulatory components of ABA) family. Therefore, CRL4-CDDD complexes are negative regulators of ABA-mediated stress responses. Conversely, ABA treatments attenuate PYL8 receptor degradation, although the precise molecular details of this mechanism remained unknown. Here, we show that ABA promotes the disruption of CRL4-CDDD complexes, leading to PYL8 stabilization. ABA-mediated CRL4-CDDD dissociation likely involves altered association between DDA1-containing complexes and the CSN, a master regulator of the assembly of cullin-based E3 ligases, including CRL4-CDDD. Indeed, treatments with CSN5i-3, an inhibitor of the CSN activity, suppressed the ABA effect on CRL4-CDDD assembly. Altogether, our findings indicate that ABA stabilizes PYL8 by altering the dynamics of the CRL4-CDDD-CSN complex association, unveiling a regulatory mechanism by which a plant hormone inhibits an E3 ubiquitin ligase to protect its own receptors from degradation.

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The adaptor protein AP-3β disassembles heat-induced stress granules via 19S RP-associated deubiquitylation in *Arabidopsis*

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Abstract

To survive under adverse conditions, plants form stress granules (SGs) to temporally store mRNA and halt translation as a primary response. Dysregulation in SG disassembly can have detrimental effects on plant survival after stress release, yet the underlying mechanism remains poorly understood. Using Arabidopsis as a model system, we demonstrated that the AP-3 subunit AP-3β interacts with the SG core RNA-binding proteins TSN1/2 in vivo and in vitro. We also showed that AP-3β is rapidly recruited to SGs upon heat induction and plays a key role in SG disassembly after heat release. Genetic evidences support that AP-3β serves as an adaptor to recruit the 19S regulatory particle (RP) of the proteasome to SGs upon heat induction. Notably, the 19S RP promotes SG disassembly through RP-associated deubiquitylation, independent of its catalytic activity. This deubiquitylation process of SG components is crucial for translation reinitiation and growth recovery after heat release. Our findings shed light on the non-catalytic function of the RP in regulating SG disassembly and highlight the importance of endomembrane proteins in supporting RNA granule dynamics in plant cells.

Key words:

stress granules; AP-3β; TSN1/2; 19S RP; deubiquitylation

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Arabidopsis inositol polyphosphate kinases regulate COP9 signalosome activities in phosphate homeostasis.

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Abstract

Cullin RING Ubiquitin E3 ligases (CRLs) facilitate targeted protein degradation during physiological development and adaptation to stress. The deneddylase activity of the COP9 signalosome (CSN) complex regulates cellular pools of neddylated cullins, required to assemble a functionally active CRL. Although, selective inositol polyphosphates (InsPs) have been implicated as co-factors in plant responses that typically require ubiquitylation of negative regulators, a direct link between any InsPs and regulation of CSN-CRL activities is not established. We identify interactions between specific *Arabidopsis thaliana* InsP-kinases and the CSN subunits. These interactions modulate the deneddylase efficiency of the endogenous CSN. Selective InsP-kinase mutants have altered cullin deneddylation rates, dis-equilibrium in CSN holo-complex composition, and defects in the association/dissociation cycles of CRLsrelated players. We further reveal that the constitutive phosphate starvation response (PSR) previously reported for these InsP kinase mutants, is caused in part by reduced stability of a key negative PSR regulator, and is suppressed by pharmacological inhibition of neddylation, thus linking CSN-CRL dynamics to phosphate (Pi)-sensing. Overall, our data reveal that the regulation of plant Pi-homeostasis and Pi-starvation responses by selective InsP-kinases is in part caused by a direct role of these kinases, in balancing coordination between CRL-CSN activities.

Keywords: Cullin-RING Ligases, inositol polyphosphates, COP9 Signalosome, phosphate homeostasis, neddylation/deneddylation.

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COP1-Mediated Ubiquitination of the Salicylic Acid Receptor: A Key Mechanism in Regulating Plant Immunity

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Abstract

CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), a key E3 ubiquitin ligase in photomorphogenesis, is known to target substrates for degradation in various biological processes. However, its role in the ubiquitination and degradation of phytohormone receptors remains largely unexplored. *Arabidopsis* NONEXPRESSER OF PR GENES 1 (NPR1) and its paralogs, NPR3 and NPR4, bind salicylic acid (SA) and mediate SA signaling. While NPR1 regulation is well understood, how NPR3 proteins are regulated in immune responses remains largely unknown. Here, we show that NPR3 stability decreases during systemic acquired resistance (SAR). COP1 directly interacts with NPR3 and ubiquitinates it at Lys237, Lys400, Lys494, and Lys552, promoting its degradation during SAR. This ubiquitination also significantly reduces the binding affinity of NPR3 for salicylic acid (SA), thereby limiting NPR1 degradation by NPR3. Notably, mutation of COP1 completely abolishes SAR. Our genetic analysis further shows that COP1-mediated immune activation is almost entirely dependent on NPR3 function. Furthermore, during late-stage SAR, excess NPR1 regulates its own levels by inhibiting COP1-mediated NPR3 degradation. Overall, our study identifies COP1 as a novel regulator in SAR, the first E3 ligase targeting the salicylic acid receptor NPR3, and reveals a mechanism by which ubiquitination affects receptor stability, ligand binding, and NPR1 self-regulation.

Key words: COP1, NPR3, NPR1, Salicylic Acid, 26S proteasome

Highlights:

1. COP1 is the first identified E3 ligase targeting the salicylic acid receptor NPR3.
2. COP1 ubiquitinates NPR3, promoting its degradation and reducing its SA-binding affinity.
3. COP1 acts as a novel positive regulator in systemic acquired resistance (SAR).
4. A novel mechanism by which excessive NPR1 achieves self-regulation: through the inhibition of COP1-mediated ubiquitination and degradation of NPR3.

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The fungal COP9 signalosome and the velvet domain transcriptional regulators coordinate development and secondary metabolism in *Aspergillus nidulans*

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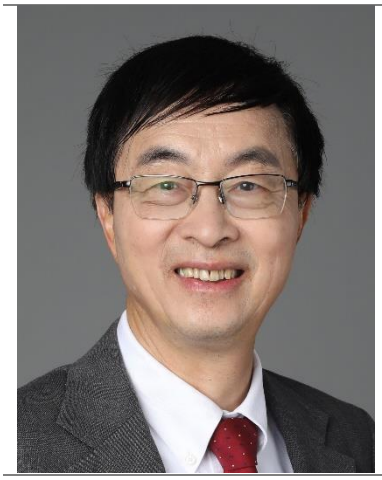
Abstract

The fungal COP9 signalosome (CSN) is required to coordinate multicellular fungal development with the appropriate secondary metabolism as chemical language to communicate with the environment. CSN is assembled by the connection of two trimeric intermediates, which are connected by Csn2/B to a hepatmeric pre-CSN, which is activated by the incorporation of the deneddylase. The coordinated fungal development and secondary metabolism requires 8 of 74 F-box proteins of the mold *Aspergillus nidulans*, which are located in the nucleus. The velvet domain transcription factors resemble in their structure the mammalian NF-kappa B regulators and are controlling fungal development. Accurate surveillance of the protein stability and cellular localization of regulators as the velvet domain transcription factors is prerequisite of fungal differentiation. The current studies of our studies of this interplay will be discussed.



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Targeting protein neddylation and Cullin-RING ligase for anti-cancer therapy

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Abstract

Protein neddylation is catalyzed by three-enzyme cascade, including E1 neddylation activating enzyme (NAE, E1), E2 conjugating enzyme, and E3 ligase. The cullin family members are physiological substrates of neddylation, and cullin neddylation is required for activation of cullin-RING ligases (CRLs). In various types of human cancers, the neddylation pathway is abnormally activated, leading to overactivation of CRLs. We have validated that the neddylation E2 UBE2F and E3 SAG (also a dual E3 for ubiquitylation) are promising therapeutic targets in lung and pancreatic cancers.

Through the structure-based virtual screen, and an alpha-screen-based high-throughput screen (HTS), followed by chemical optimization via SARs (Structure Activity Relationships), we discovered three classes of small molecule inhibitors targeting neddylation E1, E2 or E3, respectively. We also performed DEL (DNA-encoded library) screening to identified small molecules that either inhibit SAG ligase activity for CUL5 neddylation or bind to SAG-CUL5 complex as the potent ligands for PROTAC application. My presentation will summarize our results and current efforts towards the discovery of novel classes of small molecules inhibitors targeting neddylation-CRLs.

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Understanding the Structure and Function of the COP9 Signalosome Through Protein-Protein Interactions

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Abstract

The COP9 signalosome (CSN) is essential for cellular and developmental processes in animals and plants, and functions as a deneddylase by removing Nedd8 modifications from cullin proteins within Cullin–RING ubiquitin E3 ligases (CRLs). The activation and function of the CSN rely on its structural dynamics, and the binding of neddylated CRLs to the CSN can induce significant conformational changes. Despite intensive studies, it remains unclear how CSN can modulate its topology to interact with hundreds of substrates in cells for its activation and function. In order to unravel molecular details underlying CSN structural dynamics, we have employed cross-linking mass spectrometry (XL-MS) to obtain a comprehensive interaction map of the CSN complex. Identification of cross-linked peptides permits simultaneous determination of both identities and site-specific contacts of protein interactions, thus uncovering direct interactors and yielding protein structural details at residue-level resolution. The integration of XL-MS data with AlphaFold prediction and integrative structural modeling allows us to elucidate the architectures of CSN and CSN-CRL complexes, offering new insights into their regulation through protein-protein interactions.

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Protective Role of the COP9 Signalosome in Cardiovascular and Neurovascular Disease

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Abstract

The constitutive photomorphogenesis 9 (COP9) signalosome (CSN) is a multifunctional protein signaling complex and deNEDDylase controling ubiquitination activity of cullin-RING-E3 ligases (CRLs) and thus the levels of key cellular proteins. While the CSN and its catalytic subunit CSN5 have been extensively studied in cancer, its role in cardio- and neurovascular diseases such as atherosclerosis and ischemic stroke is less understood. We previously showed that the CSN has atheroprotective functions by attenuating the inflammatory NFκB signaling pathway, which in turn regulates inflammatory cytokines such as IL-1β and TNF or chemokines such as CCL2, CXCL1, or MIF. Those studies were initially carried out in myeloid-specific *Csn5* knockout (KO) mice in an hyperlipidemic atherogenic model (*Csn5*$^{\Delta myeloid}$/*Apoe*$^{-/-}$) (Asare et al, *PNAS* 2017). We have now expanded these studies to more completely define the pathways and cell- and tissue-specificities of the atheroprotective activity of CSN5 and to identify translational strategies. Myeloid-specific deficiency of *Csn8* (*Csn8*$^{\Delta myeloid}$/*Apoe*$^{-/-}$) and artery-specific KO of *Csn5* (*Csn5*$^{\Delta arterial}$/*Apoe*$^{-/-}$) also led to a pronounced exacerbated atherosclerotic phenotype with enhanced atherosclerotic plaques as well as higher vulnerability. Of note, unbiased inflammatory array analysis and bulk RNAseq in combination with treatment by Csn5-i3 and MLN4924 identified Timp1 as a novel Csn5-dependent atheroprotective target, while the IFN/CXCL10 pathways was unraveled as a key inflammatory axis attenuated by the CSN in arterial endothelial cells. Moreover, proteomics and functional experiments in cell cultures and organotypic brain cultures together with ischemia-reperfusion challenge indicated a protective role for the CSN as well. We conclude that CSN5 and the CSN holocomplex are protective in atherosclerotic diseases, neuroinflammation and ischemic stroke. In my talk, I will discuss the experimental approaches, disease models and mechanisms in details.

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COP9 signalosome (CSN) is a substrate of autophagy

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Abstract

Quality control of proteins and protein complexes are monitored by two major proteolytic pathways: the Ubiquitin (Ub)-Proteasome-System (UPS) and selective autophagy. Both pathways are responsible for the quick elimination of mutated, overexpressed and mail-functioning protein/protein complexes before setting up irreversible damages in cells. Both are often triggered by mono-, or di- or poly-ubiquitination of targets via E3 Ub ligases. E3 Ub ligases determine specificity of the UPS as well as of selective autophagy. So far, we know only few E3 Ub ligases of selective autophagy. Recent data show several autophagy-dependent degradation pathways which target inactive or mutated large protein complexes such as 26S proteasome and CDC48/p97 termed as proteaphagy. We demonstrate that CSN is degraded under serum starvation as well as in presents of the CSN inhibitor CSN5i-3. The CSN is specifically degraded by autophagy, if it is inactivated like its related protein complex the 26S proteasome. However, it is differing in selected details. Since mutated CSN is rapidly degraded via autophagy, the data strengthen the biological relevance of autophagy-degradation of protein complexes.

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Genetics, Mechanism and Therapy for A Mendelian Syndrome and How It Might Help With Hair Loss

Xu Tan

Chinese Institutes for Medical Research

Abstract

We have previously identified mutations in a ubiquitin ligase KLHL24 that cause a genetic skin disease (Lin et al *Nature Genetics* 2016). Mutations at the start codon of KLHL24 lead to its stabilization and gain-of-function, which causes excessive ubiquitination and degradation of key skin keratin proteins. Following studies identified KLHL24 mutations also cause alopecia and dilated cardiomyopathy, manifesting a complex Mendelian syndrome and multifaceted function of KLHL24. To identify potential inhibitors of KLHL24, we applied a fluorescence-based cellular assay to screen for compounds that block the autoubiquitination activity of KLHL24. One of the hit compounds showed capacity to rescue the excessive degradation of KLHL24 substrates, both *in vitro* and *in vivo*. Application of this compound to a knock-in mouse model of KLHL24 mutation showed significantly enhanced hair retention. This provides a proof-of-concept of a cure for the Mendelian syndrome. In addition, given that KLHL24 regulates key protein substrates in hair growth, we suspect that this compound might be helpful for alleviating hair loss in general.

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A nature-inspired HIF stabilizer derived from a highland-adaptation insertion of plateau pika Epas1 protein

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Abstract： Hypoxia-inducible factors (HIFs) play pivotal roles in numerous diseases and high-altitude adaptation, and HIF stabilizers have emerged as valuable therapeutic tools. In our prior investigation, we identified a highland-adaptation 24-amino-acid insertion within the Epas1 protein. This insertion enhances protein stability of Epas1, and mice engineered with this insertion display enhanced resilience to hypoxic conditions. In the current study, we delved into the biochemical mechanisms underlying the protein-stabilizing effects of this insertion. Our findings unveiled that the last 11 amino acids within this insertion adopts a helical conformation and interacts with the α-domain of pVHL, thereby disrupting the Eloc-pVHL interaction and impeding the ubiquitination of Epas1. Utilizing a synthesized peptide, E14-24, we demonstrated its favorable membrane permeability, and ability to stabilize endogenous HIF-α proteins, inducing the expression of HRE genes. Furthermore, administration of E14-24 to mice subjected to hypoxic conditions mitigated body weight loss, suggesting its potential in enhancing hypoxia adaptation.

Key words: Epas1, HIF stabilizer, peptide drug, α-helix

TARGETING THE E3OME

Ubiquitination-modulating proteins
and corresponding validated antibodies

Significance

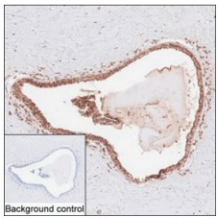
Targeted Protein Degradation (TPD) technology offers new strategies for addressing "undruggable" targets, enhancing drug selectivity and efficacy, and overcoming resistance caused by protein mutations. However, the development of this technology faces several challenges:

- Target Protein Detection
- Finding More Effective E3 Ligases
- Increasing Tissue Specificity
- Mitigating Off-Target Effects

Strengths

Leveraging our globally leading human protein library and state-of-the-art antibody development platform, Absea® has successfully developed more than 400 ubiquitination-modulating proteins constructs, and corresponding IP and IHC verified antibodies for over 50 targets. We are actively advancing the "E3ome" program, to support TDP development by providing new opportunities to screen for suitable E3 ligases and characterize their substrate spectrum.

- 400+ Ubiquitination-modulating proteins
- IP antibodies for over 50 targets
- Exclusive Magnetic Bead-Conjugated E3 IP Antibodies
- IHC antibodies for 74 targets



Cat #	Target	Tissue
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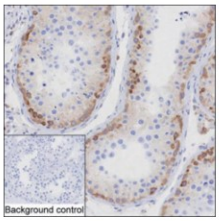

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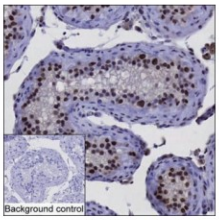

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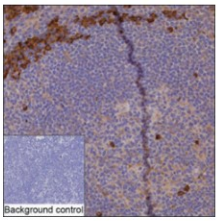

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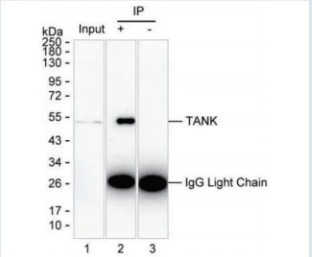

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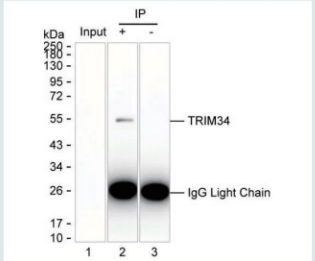

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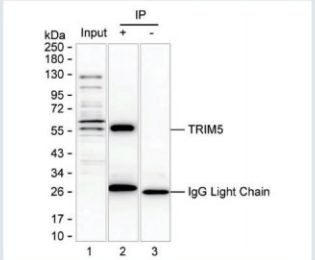

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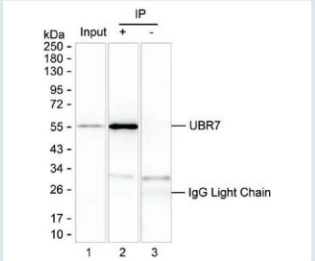

Cat #	Target
KC-2182	UBR7

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Regulation of Male Reproductive Health from an RNA Perspective

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Abstract

Male reproductive health problems have become a "silent epidemic", which greatly affects male fertility and the health of offspring. Not only that, male fertility decline is also correlated to mutiple types of human cancers and a variety of chronic diseases, and is a "barometer" of male health throughout the life cycle. As the basis of male reproductive health, spermatogenesis is the most complicated cell differentiation process in mammals, consisting of mitosis, meiosis, spermiogenesis, sperm capacitation and activation. These sequential male germline development is programmed by a series of highly orchestrated regulatory events in gene expression at each developmental step, in which RNA regulation plays an indispensable role and essential for the production of healthy sperm. Recent studies from us and others have identified causal roles of genes in multiple RNA regulatory pathways to male infertility. We have committed to the study of new functions and mechanisms of RNA regulation in spermatogenesis and male infertility. In this report, I will briefly summarize our findings on RNA regulation in spermatogenesis and male infertility, focusing on our most recent discovery of an intrinsic mechanism enabling spermatocytes to undergo the programmed meiotic progression. Inconvenience

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Structural insights into the mammalian late-stage translation initiation navigated by trans-RNAs

Longfei Jia[1], Tan-Trung Nguyen[2], Saori Uematsu[1], Yifei Gu[1], Shengcho Shi[1], Yaser Hashem[2], *, and Shu-Bing Qian[1], *

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Abstract

Translation of messenger RNA into protein is a key final step in the regulation of gene expression that is generally used to fine-tune proteostasis in the cell to regulate development, differentiation and metabolism. Diverse approaches have been developed for gene silencing, but few tools are available to activate individual mRNAs for translation inside cells. Driving translation apparatus to initiate the translation at specific start-codons without altering the original nucleotide sequence remains a challenging task. Here, we designed capped antisense trans-RNAs capable of engaging ribosomes to specific initiation sites on individual mRNAs by positioning them near the target start codon. To gain structural insights into the trans-RNA action, we attempted to purify the late-stage initiation complexes (LS48S ICs) navigated by trans-RNAs after stalling them at target start codon in near native conditions and to solve their structures by using single particle cryo-electron microscopy (cryo-EM). Our results of structural analysis provide structural snapshots of late-stage translation initiation at the specific start codons and validate the ability of trans-RNA in modulating the translation process.

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Identification and Characterization of Novel Quality Control Factors on the Ribosome

Ayala Shiber

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Abstract

The proper folding of nascent proteins is a major challenge within the crowded eukaryotic cell. At this essential intersection of translation and folding, the ribosome functions as a hub, coordinating the activities of diverse factors that guide the emerging polypeptide-chain maturation. These various factors include modifying enzymes, targeting factors and folding chaperones. Even the assembly into high-order oligomeric complexes, the final step of folding, was recently discovered to occur during protein synthesis. Co-translational assembly mechanism was found to be wide spread in the model eukaryote, *Saccharomyces cerevisiae*. Furthermore, misfolding-prone subunits were protected from aggregation and degradation by co-translational assembly interactions with their partner subunits in the complex. Leading to the question: How does the cell recognize protein misfolding even before the end of synthesis? Our objective is to identify and characterize assembly-specific degradation and sequestration factors that act in a co-translational manner. Utilizing a combination of proteomics and ribosome profiling approaches, we were able to discover a novel quality-control pathway for complex subunits that fail to assemble into functional complexes. This provides us, for the first time, direct data on a proteome-wide scale, of factors safeguarding the cellular proteome during synthesis. This research can pave the way for development of potential healthcare strategies of disease conditions characterized by protein misfolding, such as Parkinson's and Alzheimer's.

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5’-UTR-mediated tethering of eIF3 to 80S ribosomes promotes co-translational protein folding of ER membrane proteins

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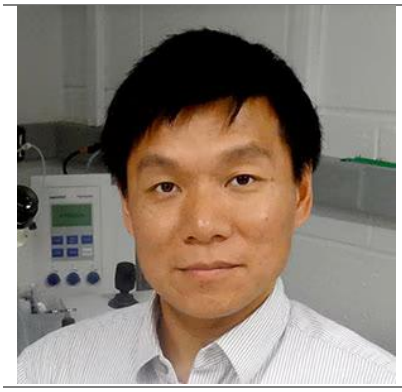
Abstract

Cellular homeostasis is crucially dependent on the correct, co-translational folding of nascent peptides into functional proteins. Misfolded proteins can form toxic aggregates that cause various age-related diseases including cancer, neurodegeneration and metabolic disease. Co-translational folding is carried out by an elaborate network of chaperones strategically positioned in the vicinity of the ribosome, but how chaperone recruitment to ribosomes is coordinated with translation initiation such as to enable timely folding of emerging nascent chains, remains poorly understood.

eIF3 is a 13-subunit protein complex that is involved in many forms of canonical cap-dependent and non-canonical translation initiation, it is now known to be retained on 80S ribosomes during early translation of certain mRNAs encoding proteins with membrane-associated functions. We report that binding of eIF3 to mRNA 5'-UTRs promotes its retention on post-initiation 80S ribosomes translating mRNAs encoding membrane-associated proteins. Disrupting the eIF3-80S ensemble by disabling eIF3 5’-UTR binding sites leads to the misfolding of newly synthesized proteins. Misfolding is exacerbated when HSP70 chaperones are inhibited but rescued by overexpressing HSPA8 and HSPA1, chaperones recruited to ribosomes in an eIF3-assisted manner. Thus, the instructions for co-translational protein folding of ER membrane proteins are hard-wired in the genome through 5'-UTR sequences and are conveyed to 80S ribosomes via eIF3-mediated chaperone recruitment.

Key words: co-translational protein folding, eIF3, membrane-associated proteins



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NEMF-mediated CAT-tailing in ribosome-associated quality control at the endoplasmic reticulum

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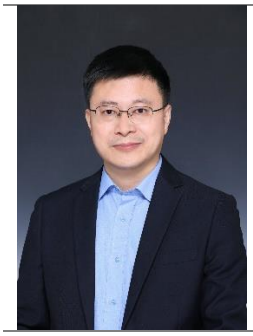
Abstract

Ribosome stalling during co-translational translocation at the endoplasmic reticulum (ER) causes translocon clogging and impairs ER protein biogenesis. Mammalian cells resolve clogged translocons vial an incompletely characterized ribosome-associated quality control (RQC) pathway that depends on the modification of ribosomal protein RPL26 with ubiquitin fold modifier 1 (UFM1), a process named ribosome UFMylation. Here, we combined genome-wide CRISPR screen with live cell fluorescence confocal microscopy to dissect the molecular linchpins of ER-RQC. We show that substrates translated from mRNAs bearing a ribosome stalling poly-(A) sequence can be degraded by lysosomes and proteasome. By contrast, the degradation of an ER-RQC substrate encoded by non-stop mRNA is mediated by an unconventional ER-associated degradation (ERAD) mechanism that involves ER-to-Golgi trafficking and KDEL-dependent ER retrieval of non-stop substrate. The diversity in triaging option appear to result from the heterogeneity of NEMF-mediated CATylation, as an AT-rich tail can serve as a degron for ERAD, while an AG-rich tail allows an otherwise secretory protein to be sorted to lysosomes or retrieved back to the ER from the Golgi. Thus, our study reveals an unexpected protein triaging function of CAT-tailing that acts in conjuction with ribosome UFMylation to safeguards protein biogenesis at the ER.



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Applications of chemical protein synthesis in studying ubiquitin biochemistry

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Abstract

The chemical synthesis of proteins can help prepare proteins that are difficult to obtain using recombinant techniques, such as proteins carrying complex post-translational modifications, proteins containing multiple unnatural amino acids, proteins with non-linear structures, and mirror image proteins, expanding the ability to create new substances. We have developed a method system for protein chemical synthesis based on protein and peptide hydrazides, including peptide hydrazide solid phase synthesis, protein hydrazide recombinant expression, multi-segment hydrazide convergent ligation, protein backbone modification, protein glycosylation-assisted folding, etc. Using the method system we have achieved the chemical synthesis of more than 100 proteins such as polyubiquitin, modified histones, cytokines, GPCR, and nucleic acid polymerases, and the use of chemically synthesized proteins has generated more and more applications in biochemical mechanisms and biomedical research. As a representative case, our team investigates the use of protein chemical synthesis to resolve the biochemical and structural mechanisms of protein ubiquitination-related enzyme complexes, especially pathology-related ubiquitin E3 ligases and deubiquitinating enzymes.

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Regulating constitutive activation of NF-κB signaling by ubiquitin ligase BRAP

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Abstract

NF-κB is a family of transcription factors which mediate inflammation. It can be activated by many extracellular and intracellular stimuli, such as cytokines, growth factors, mitogens and infectious agents etc. In resting states, NF-κB is restricted in cytosol by inhibitory proteins, such as IκBα. Upon stimulation, IκBα is often inactivated via ubiquitylation by ubiquitin ligase $SCF^{\beta TrCP}$ and proteolysis by the 26S proteasome, thereby releasing NF-κB into nuclear where it turns on transcription of many inflammation-related genes. NF-κB activation is often a transient event since there are many inhibitory mechanisms in normal cells. In contrast, NF-κB is often constitutively activated in many cancer cells, including basal-like breast cancer. However, the underline mechanism is still unclear. Here we show that IκBα, as a main NF-κB inhibitor, is an unstable protein and degraded by the ubiquitin-proteasome pathway in basal-like breast cancer (BLBC) and pancreatic cancer cells. Moreover, we identified BRAP as a ubiquitin ligase which controls the constitutive degradation of IκBα and is responsible for expression of many inflammation-related genes in BLBC. Furthermore, we found that BRAP-mediated IκBα proteolysis is independent of the SCF ubiquitin ligase and the classic phosphorylation events leading to NF-κB activation. Our data suggested a new NF-κB signaling pathway exist in BLBC and other inflammatory cancer cells. This new pathway could provide potential targets for therapies of BLBC and other inflammatory cancers.

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A fourth proteasome-associated DUB?

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Abstract

There are nearly 100 deubiquitinating enzymes (DUBs) encoded by the human genome, but only three are currently considered proteasome-associated DUBs (pDUBs): Rpn11/PSMD14, Ubp6/USP14, and UCH37/UCHL5. Of these, only Rpn11/PSMD14 is an integral subunit of the proteasome, positioned deep within the 19S regulatory particle (RP), whereas the other two transiently dock at dedicated peripheral proteasomal subunits. Specifically, UCHL5 interacts with ADRM1-PSMD1, and USP14 associates with PSMD2 via its N-terminal ubiquitin-like domain (UBL). Additionally, proteasome function is often modulated by these or other transiently associated proteins.

Given that proteasome composition is dynamic and some pDUBs are transient, we predicted that additional UBL-containing DUBs might be associated with proteasome complexes. Analysis of proteasome composition from different sources identified a fourth DUB intimately associated with the proteasome through its UBL domain. To understand and characterize this association, we employed biochemical methods and biomolecular approaches to elucidate the purpose of this interaction and its importance in regulating proteasome activity. Our results reveal that this pDUB helps discriminate between substrates by rapidly disassembling short polyubiquitin chains but not K48-linked tetra-ubiquitinated substrates, thus aiding in the selection of substrates that are deconjugated and repelled from those committed to proteolysis.

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Regulation of the ATR-CHK1 checkpoint signaling

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Abstract

To maintain genome integrity upon replication stalling at damaged template strands, cells have evolved a complex mechanism known as the S phase checkpoint to detect and repair DNA damage that occurs during replication. This checkpoint is mediated by the ATR (ataxia telangiectasia and Rad3-related protein)-CHK1 (checkpoint kinase 1) pathway. CLASPIN is an essential mediator for ATR-dependent CHK1 activation upon replication stress. We have identified several novel regulatory mechanisms for this checkpoint activation, including ubiquitination/deubiquitination of CLASPIN, PARP1 UFMylation, and ASPM (a protein encoded by primary microcephaly 5)-dependent recruitment of RAD9/TopBP1. Our works have further elaborated the complicate yet delicate regulatory networks for replication fork stability during replication stress, thus safeguarding genome integrity.

Key words: ubiquitination/deubiquitination, UFMylation, checkpoint signaling, ATR-CHK1

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A nuclear Protein Quality Control system for the elimination of nucleolus-related inclusions

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Abstract

The identification of pathways that control the elimination of protein inclusions is essential to understand the cellular response to proteotoxicity, particularly in the nuclear compartment for which our knowledge is limited. We report that stress-induced nuclear inclusions related to the nucleolus are eliminated upon stress alleviation during the recovery period. The process is independent of the autophagy/lysosome and the CRM1-mediated nuclear export pathways, but strictly depends on the Ubiquitin UBA1 E1 enzyme and nuclear proteasomes that are recruited within the formed inclusions. Strikingly, UBA1 activity is essential only for the recovery process but dispensable for nuclear inclusion formation. The HUWE1 E3 ligase and HSP70 are the components of the Ubiquitin/Chaperone systems that promote inclusion elimination. The recovery process also requires the RNA Pol I dependent production of lncRNAs. We defined a class of lncRNAs that are specifically induced upon stress conditions and localise within the formed inclusions and promote their elimination by preserving the mobility of resident proteins. The study reveals a Protein Quality Control system that operates within the nucleus for the elimination of stress-induced nucleolus-related inclusions.

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Characterization of the ubiquitination code mediated by Parkin in the course of mitophagy

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Abstract

Mitophagy is a selective form of autophagy for clearance of damaged mitochondria via the autophagy-lysosome pathway. Among various mitophagy regulatory mechanisms, PINK1, a protein kinase, and Parkin, an E3 ligase, are two critical players, with important implications in neurodegenerative disorders such as Parkinson's disease (PD). In our recent studies, we have attempted to characterize the unique features of the ubiquitination code mediated by Parkin in the course of mitophagy downstream of PINK1. In this presentation, I will present some of our unpublished data on (i) the functional implication of proteasome in mitophagy, (ii) the type and length of the ubiquitin chains mediated by Parkin in response to acute mitochondrial damage, and (iii) the role of p97/VPS complex and the effect of complex on recruitment of mitophagy cargo receptors such as OPTN and NDP52. The results from the above-mentioned studies provide insights into the molecular mechanisms of mitophagy and lay foundation for development of interventional strategies for mitophagy-related human diseases such as neurodegeneration and cancer.

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CCT7 functions as monosome for clearance of ALS- related aggregates

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Abstract

ALS is a devastating motor neuron disease that leads to progressive muscle weakness and paralysis, with no current cure. The chaperonin-containing TCP-1 (CCT) complex, which is involved in maintaining protein homeostasis, may affect the onset and progression of ALS, but the specific molecular mechanisms are unclear. Recent studies have shown that CCT subunit CCT2 act as monomer to clear aggregated proteins through autophagy named aggrephagy. Our investigations also found CCT7 could form aggresome-like structure with its monomer status, in which enriched of ALS- related proteins. Besides, the aggresome-like structure were partially colocalized with LC3B, a marker of autophagy. Additionally, CCT7 have been found to be expressed in motor neurons and partially co-localize with the ALS-associated protein SOD1 in the spinal cord of ALS mice. Based on these findings, it is speculated that CCT may regulate the expression of ALS-related proteins through aggrephagy as well, thus influencing the onset and progression of ALS. These findings provide potential novel targets for understanding and treating ALS, offering new insights for ALS therapy.

Key words: Chaperonin, CCT monomer, Aggrephagy, ALS

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Protein Quality Control Regulates Stress Granule Homeostasis

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Abstract

Stress granules (SGs) are membraneless organelles formed in response to cellular stress, and they play a key role in protecting cells by sequestering and regulating stress-related molecules. Disruptions in SG dynamics have been linked to neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). In our previous study, we demonstrated high-resolution, time-resolved proteomic profiling to track the compositional and functional transitions throughout the SG life cycle and provided insights into how SGs achieve their diverse, stress-type-specific functions (Hu et al., Nature Communications, 2023). Furthermore, we investigated protein quality control mechanisms regulating SG homeostasis under various stress conditions (Yang et al., Autophagy, 2023). In this study, we delved into the detailed mechanisms governing the autophagic degradation of high salt-induced SGs and SG-colocalized pathological inclusions. We established a high salt-induced SG model and identified LC3 isoform A (LC3A) as the key protein mediating the autophagic degradation of these SGs. Through proteomic analysis of affinity-purified SGs under prolonged high salt conditions, coupled with comprehensive immunostaining and genetic analysis, we discovered that LC3A specifically colocalizes with SGs and facilitates their degradation. The SG proteome also revealed various key autophagy-related proteins, including the autophagy receptor SQSTM1/p62 and the segregase VCP, whose depletion markedly compromised SG degradation. Additionally, LC3A-mediated degradation was critical for clearing pathogenic inclusions formed by disease-associated variants. These findings highlight the pivotal role of the SQSTM1-LC3A axis in the autophagic degradation of SGs under chronic stress and pathological conditions, providing new insights into the cellular stress response and its implications for diseases.

Key words: Amyotrophic lateral sclerosis (ALS), autophagy, LC3A, SQSTM1/p62, stress granule, ubiquitin

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Multi-dimensional analyses reveal antagonism between JAK/STAT downstream targets UBR5 and Drumstick in stem cell regulation and cell fate conversion

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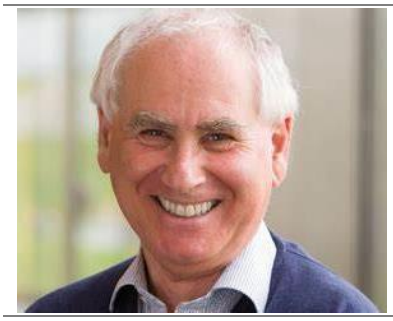
Abstract

Most, if not all, stem cells reside in the niche. Different cell types within the niche must maintain their specialized cell fates and cell fate conversions between different cell types are strictly controlled to maintain the steady-state of a stem cell niche. However, how cell fate conversion is regulated within a stem cell niche remains poorly understood. Here, we systemically identify JAK/STAT downstream targets in adult *Drosophila* testis by multi-omics approaches. *ubr5*, encoding an E3 ligase in the N-end rule pathway, is identified as one putative JAK/STAT target. Depletion of *ubr5* in somatic cyst cells cell-autonomously affects the proliferation and differentiation of cyst stem cells (CySCs) and non-cell autonomously affects germline stem cell (GSC) proliferation and differentiation. Importantly, *ubr5-defective* CySC-like cells convert their fate into hub cell fate. Mechanistically, UBR5 directly interacts with Drumstick (Drm), another putative JAK/STAT target, through its UBR domain and mediates Drm poly-ubiquitination for proteolysis. Ectopic expression of drm mimics *ubr5*-depleted testes and further removal of *drm* completely suppresses the defects observed in *ubr5*-depleted testes. Finally, the function of UBR5 is evolutionarily conserved. Collectively, UBR5 antagonizes Drm to control JAK/STAT signaling duration, stem cell proliferation, differentiation, and cell fate conversion within the testicular niche. Thus our study uncovers the underlying mechanism of how the steady-state operation of a stem cell niche is properly maintained by JAK/STAT signaling.



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MECHANISM OF PROTEIN IMPORT INTO PEROXISOMES

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Abstract

Peroxisomes are ubiquitous organelles whose dysfunction causes fatal human diseases. Most peroxisomal enzymes are imported in a folded state from the cytosol by the receptor PEX5. Recent work shows how PEX5 shuttles cargo into peroxisomes. PEX5 binds cargo in the cytosol and then enters peroxisomes by a process resembling nuclear transport. A meshwork is formed inside the membrane by a conserved tyrosine/glycine-rich YG domain of PEX13, and resembles the meshwork of nucleoporin FG domains inside nuclear pores. PEX5 selectively partitions into this phase, using conserved aromatic motifs, and brings bound cargo along. PEX5 returns to the cytosol through a retro-translocon formed by a ubiquitin ligase complex, consisting of PEX2, 10, and 12. The ligase complex has an open pore, into which the import receptors insert a flexible N-terminal segment from the lumenal side. Following mono-ubiquitination, PEX5 is pulled out of peroxisomes by the PEX1/6 ATPase. During retro-translocation, PEX5 is unfolded, which results in cargo release inside the organelle. After folding and deubiquitination, PEX5 can start a new import cycle. Recent results clarify how proteins with an N-terminal PTS2 signal are imported into peroxisomes. These proteins use the adaptor PEX7 to bind to a receptor. After import, PEX7 partitions into the YG phase from the lumenal side and is extracted by PEX39 back into the cytosol.

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A Non-E3 Function of Ubiquitin Ligase HERC3

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Abstract

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FAST is a fatty acid-sensing/transducing E3 complex that safeguards lipid droplet homeostasis to prevent lipotoxic disorders

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Abstract

Fatty acid (FA) imbalance and ectopic deposition are increasingly appreciated to be the key cause of metabolic disorders such as obesity, diabetes, and MASLD. Yet it remains unclear how cells monitor and dispose toxic cytosolic free FAs (FFA). We discovered FAST, a fatty acid-sensing/transducing ubiquitylation E3 complex, that directly senses fatty acids to gate lipid droplet (LD) growth. The basal FAST complex restricts lipid droplet expansion by ubiquitylating the acyl-CoA synthetases FATP1/4 and preventing their coupling with the triglyceride (TG) synthase DGAT2. Upon FA incubation, cytosolic unsaturated FAs compete with FAST subunit a (FASTa) to bind FAST subunit b (FASTb), leading to FAST disassembly and enhanced FATP-DGAT2 coupling for TG synthesis and storage. FAST-depleted cells display accelerated LD growth, whereas mice with hepatocyte-specific FAST subunit deletion display exaggerated fatty liver and hepatocellular carcinoma development. Our study provide a molecular basis of intracellular FA-sensing by delineating a FA-relievable ubiquitylation checkpoint that keeps LD dynamics in sync with FFA levels.

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The role of ubiquitin ligase MARCH5 in peroxisome homeostasis

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Abstract

Ubiquitination plays an important role in organelle quality control. MARCH5 is a mitochondrion-associated ubiquitin ligase, best known for regulating mitochondria dynamics and protein quality control. Recently, our group and Dr. Matsuda's group independently reported the dual organelle localization of MARCH5 (PMID: 34747980, 31602805). In our prior work, we demonstrated that the presence of peroxisomal MARCH5 is essential for mTOR inhibition-induced pexophagy (PMID: 34747980, 34889952).

In this study, we investigate the role of mitochondrial MARCH5 in peroxisome biogenesis. By generating a cellular model for peroxisome *de novo* biogenesis from mitochondria, we observed the localization of peroxisomal proteins to mitochondria and the generation of newly synthesized peroxisomes from mitochondria via mitochondria-derived vesicles. We find MARCH5 is essential for this process, and the loss of MARCH5 specifically impedes the budding of PEX3-containing vesicles from mitochondria, thereby blocking the formation of pre-peroxisomes. We further demonstrate that this process is both MARCH5 and PEX3 specific. Overall, our study highlights a novel function of MARCH5 for mitochondria derived pre-peroxisomes, emphasizing MARCH5 as one key regulator to maintain peroxisome homeostasis.

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Molecular basis for the stepwise and faithful assembly and maturation of the 20S proteasome

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Abstract

The proteasome degrades most of the excess and damaged soluble proteins in cells, and its decline is associated with many diseases. As the proteolytic unit of this gigantic complex, the 20S proteasome contains 28 subunits, which are assembled into a barrel-shape particle under the assistance of five chaperone proteins PAC1/2/3/4 and POMP. The assembly is followed by a maturation process that activates the proteolytic sites and clears the chaperones that blocks the proteolytic chamber. However, the molecular mechanisms underlying the 20S proteasome assembly and maturation remain to be learned. We established a method to capture a series of low-abundance and highly dynamic assembly intermediates of the human 20S proteasome. Their high-resolution three-dimensional structures were resolved by cryo-electron microscopy. Through further structural and functional studies, we identified key molecular switches for the proteolytic-site activation, revealed the mechanism precisely controlling the clearance of the assembly chaperones, and uncovered the checkpoints safeguarding transitions between crucial assembly steps. Taken together, our study elucidates the molecular mechanisms mediating the stepwise and faithful biogenesis of the 20S proteasome and lays the foundation for building more proteasomes to combat the decreased capacity of protein degradation in aging and disease.

Key words: Protein degradation, Proteasome, 20S proteasome, Assembly, Maturation

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Direct proteasomal degradation of target proteins by non-ubiquitin PROTAC, NuTAC

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Proteolysis targeting chimera (PROTAC) technology uses heterobifunctional protein degrader molecules consisting of a target-protein binder and a ubiquitin ligase binder. Once the target protein and ubiquitin ligase are brought into close physical proximity, the target protein is ubiquitinated by the ubiquitin ligase and then degraded by the ubiquitous 26S proteasome. But there are more than 600 different ubiquitin ligases, which are regulated extensively and sophistically, adding tremendous uncertainty to the success of PROTAC approaches. We show here that the target protein can be brought to Rpn13, a substrate receptor subunit of the 26S proteasome, by a heterobifunctional small molecule, and is then degraded by the proteasome independently of its ubiquitination. We have obtained a small molecule ligand that binds Rpn13 following a high-throughput screening. After linking this Rpn13 ligand to the molecule binding the target protein BRD4 or PD-L1 to form a tripartite non-ubiquitin proteolysis targeting chimera (NuTAC), the target degradation can be triggered by this NuTAC molecule, leading to potent tumor repression in mice. Thus, NuTAC might target any cellular proteins for degradation using only one proteasome-binding ligand without reversible ubiquitination.

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Mini-PROTACs

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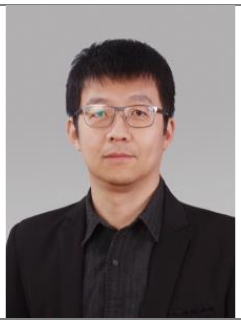
Abstract

Proteolysis-targeting chimera (PROTAC) that selectively eliminates detrimental proteins by exploiting the ubiquitin-proteasome system (UPS) represents a promising therapeutic strategy for various diseases. Effective adaptions of degradation signal sequences and E3 ligases remain limited. We have developed a set of single amino acids-based PROTACs to target oncoproteins for degradation by the N-end rule pathway. Single amino acids, the first and simplest degradation signals identified in 1980s, can be easily changed to bring speed control for PROTACs. We have employed distinct amino acids as the ligand to recruit the corresponding E3 ligases CRL2, GID4 and UBRs to degrade target proteins. We found that the extent of target reduction can be easily fine-tuned with different amino acids. These amino acids based PROTACs hindered cancer cell proliferation and induced the cell cycle arrest and apoptosis *in vitro*, and blocked the tumor growth in a xenograft tumor model *in vivo*. Comparing to other PROTACs, these PROTACs developed are small, interchangeable but with different degradation efficiency and further expand the E3 ligases and their ligands repertoire for PROTAC application, improving the versatility and utility of target protein degradation for therapeutic purposes.



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C-degron-mediated proteostasis and its implication in PROTAC design

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Abstract

Ubiquitin-proteasome system (UPS) acts as the major route for protein degradation in higher eukaryotes. A degron serves as a transferable destabilizing signal targeting an E3 ligase to mediate PolyUb and subsequent proteasomal proteolysis of the substrate. The E3-degron interaction determines the specificity of UPS. Previously, we uncovered the mechanism underlying Arg/C-degron recognition by FEM1B, a substrate receptor of Cullin 2-RING ligase (CRL2). Recently, we discovered that FEM1B prefers C-degrons harboring both a C-terminal Pro and an upstream aromatic residue (Ψ). By solving several C-degron-bound structures of $CRL2^{FEM1B}$, we elucidate distinct dimerization states of unmodified and neddylated $CRL2^{FEM1B}$ E3 ligase, as well as the Ψ-Pro/C-degron recognition by FEM1B. In addition to FEM1B, we also revealed the R-x-x-G/C-degron recognition by APPBP2, another CRL2 substrate receptor. Through compound screening, we discovered that 13-B10 directly binds to the C-degron binding pocket of FEM1B, implying its good potential in PROTAC design for targeted protein degradation.

Key words: Ub-Proteasome system, 26S proteasome, Cullin-RING E3 ligase, Substrate receptor, C-degron

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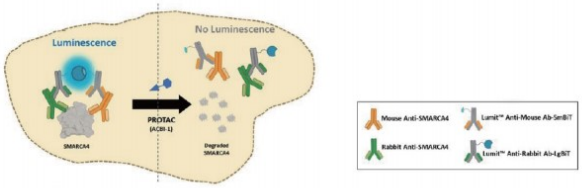

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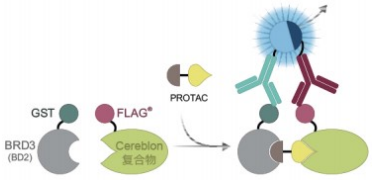

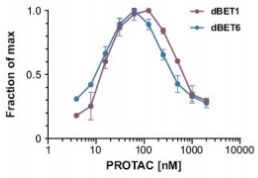

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USP15: a fourth Proteasome-associated DUB

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Abstract

The human genome encodes nearly 100 deubiquitinating enzymes (DUBs), but only three are considered proteasome-associated DUBs (pDUBs): Rpn11/PSMD14, Ubp6/USP14, and UCH37/UCHL5. Among these, only PSMD14 is an integral 19S subunit of the proteasome. UCHL5 and USP14 transiently bind to specific proteasomal subunits, with UCHL5 interacting with ADRM1-PSMD1 and USP14 associating with PSMD2 via its N-terminal ubiquitin-like domain (UBL). Given the dynamic nature of proteasome composition and the transient role of some pDUBs, we sought to identify additional DUBs associated with proteasome complexes. Analysis of proteasome composition across various sources indicated that USP15 is intimately associated with the 26S proteasomes. In proteasomes preparations purified from erythrocytes, USP15 was identified as the second most abundant pDUB. We employ biochemical and biomolecular methods to study this interaction, finding that USP15 binds specifically to PSMD2 through one of its UBL domains. At the proteasome, USP15 facilitates substrate discrimination by efficiently disassembling short polyubiquitin chains, while sparing K48-linked tetra-ubiquitinated substrates, thereby aiding in substrate selection for deconjugation versus proteolysis.

Key words: 26S proteasome, DUBs, proteasome associated proteins, Ubiquitin.

Super enhanced purification of denatured-refolded ubiquitinated proteins by tandem hybrid ubiquitin binding domain (ThUBD)

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Ubiquitination is an important post-translational modification that maintains protein homeostasis and participates in multiple biological processes. It is of great scientific significance to profile ubiquitinome. Due to the low abundance of ubiquitinated proteins, the first and vital step is to enrich ubiquitinated proteins efficiently. Currently, the antibody or artificial ubiquitin-binding domains (UBDs) are commonly used to enrich ubiquitinated proteins under native condition. The enrichment depend on the naturally spatial structure of ubiquitin and ubiquitin chains. However, the high activity of deubiquitinating enzymes and proteasomes make it easy for the ubiquitination signal to be removed, and a large number of contaminant proteins are present. In this study, we propose a new method to enrich ubiquitinated proteins by denatured-refolded ubiquitinated sample preparation (DRUSP) coupled with ThUBD. The proteins were sufficiently extracted under denaturation conditions, and the spatial structure of ubiquitin and chains were quickly and accurately restored. ThUBD could efficiently enrich the modified substrates. The overall enrichment effect of ubiquitin signal is increased by about 2.4 folds, the average enrichment effect of 8 ubiquitin chains is increased by about 10 folds. The stability and reproducibility of quantitative identification by the new method are obviously improved.

Key words: DRUSP, Denatured-Refolded, ThUBD, Ubiquitinomics, MS

Structure-Guided Engineering Enables E3 Ligase-Free and Versatile Protein Ubiquitination via UBE2E1

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Ubiquitination, catalyzed usually by a three-enzyme cascade (E1, E2, E3), regulates various eukaryotic cellular processes. E3 ligases are the most critical components of this catalytic cascade, determining both substrate specificity and polyubiquitination linkage specificity. Here, we reveal the mechanism of a naturally occurring E3-independent ubiquitination reaction of a unique human E2 enzyme UBE2E1 by solving the structure of UBE2E1 in complex with substrate SETDB1-derived peptide. Guided by this peptide sequence-dependent ubiquitination mechanism, we developed an E3-free enzymatic strategy SUE1 (sequence-dependent ubiquitination using UBE2E1) to efficiently generate ubiquitinated proteins with customized ubiquitinated sites, ubiquitin chain linkages and lengths. Notably, this strategy can also be used to generate site-specific branched ubiquitin chains or even NEDD8-modified proteins. Our work not only deepens the understanding of how an E3-free substrate ubiquitination reaction occurs in human cells, but also provides a practical approach for obtaining ubiquitinated proteins to dissect the biochemical functions of ubiquitination.

Key words: Polyubiquitination, E3-independent ubiquitination, Branched ubiquitin chains

C-mannosyltransferase DPY19L1L mediated Reissner Fiber assembly is critical for zebrafish body axis straighten

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Protein C-mannosylation potentially provides additional adhesion functions. DPY19L1 (dumpy-19 like 1) is a C-mannosyltransferase preferentially modifies the first two tryptophans of WxxWxxWxxC motifs, but a direct involvement in vertebrates development has never been demonstrated. We reveal here that *dpy19l1l* (dumpy-19 like 1 like) expresses in the presumptive spinal cord region during early stages of the zebrafish embryos development, and mutation of *dpy19l1l* leads to body axis curvature in the absence of muscle and cilia defects. Urotensin related peptide 2 (urp2) is downregulated in *dpy19l1l*$^{-/-}$ mutant zebrafish. Consistent with RF carried epinephrine in cerebrospinal fluid (CSF) is required for *urp* expression, treating *dpy19l1l*$^{-/-}$ mutants with epinephrine rescued the expression of the *urp* genes and body axis curvature. By providing URP peptides, we rescued body axis curvature of *dpy19l1l*$^{-/-}$ mutants during larval stages. The immunostaining result of SCO-Spondin indicated that the *dpy19l1l*$^{-/-}$ mutants were lack of RF, and the RF could be reestablished by injection of *dpy19l1l* mRNA. More strikingly, we showed here that by injection of presumptive C-mannosylation catalytic site mutated *dpy19l1l* mRNA (E106A *mdpy19l1l*) fail to RF formation. These findings suggest that DPY19L1L mediated SCO-Spondin C-mannosylation is a critical supplement mechanism underlying body axis straightening and the pathology of idiopathic scoliosis.

Key words: Zebrafish, DPY19L1L, Protein C-mannosylation, Idiopathic scoliosis

A General Strategy to Trap Enzymatic Working States of E3- and DUB-related Ub Transfer Processes

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Modulation of target protein substrate homeostasis by a E3- or DUB-regulated ubiquitination is an emerging regulatory tool and therapeutic approach. It is attractive to understand the dynamic catalytic and recognitive mechanism how a ubiquitin (Ub) is driven "on" or "off" substrates by E3s or DUBs. Efforts are focused on using chemically designed near-native Ub to form mimetic E3- and DUB-related transfer intermediates, which can be used in biophysical studies, including cryogenic electron microscopy, H/D exchange, NMR, etc., to reveal the enzymatic mechanism of E3s and DUBs. This presentation will introduce a general strategy using a bifunctional molecular auxiliary to easily prepare desired Ub analogs at a multimilligram scale. And we also would like to discuss some ongoing work resulting from this strategy that novel Ub transfer mechanisms and recognition captured at discrete stages in these processes.

Key words: ubiquitination, chemical probes, enzymatic mechanism, E3 ligase, DUB

Molecular glues targeting splicing factor RBM39 improves PARP inhibitor response in high-grade serous ovarian cancer

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High-grade serous carcinoma (HGSC) is the most common subtype of ovarian cancer with limited therapeutic options and a poor prognosis. In recent years, poly-ADP ribose polymerase (PARP) inhibitors have demonstrated significant clinical benefits, especially in patients with *BRCA1/2* mutations. However, acquired drug resistance and relapse are major challenges. In this work, we found that RBM39 mainly modulates alternative splicing of genes involved in DNA repair, DNA replication, and cell cycle. RBM39 can be depleted with a group of molecular glues such as indisulam (E7070), which degrade RBM39 through DCAF15 E3 ubiquitin ligase. Indisulam-mediated RBM39 depletion causes splicing defects in key DNA damage repair genes, including *ATM*, *BRCA1*, and *TP53BP1*, leading to reduced DNA damage repair, cell cycle arrest, and synergy with cisplatin and multiple PARP inhibitors. Notably, *in vivo* results showed that indisulam outperforms olaparib in mice bearing PARP inhibitor-resistant tumours, and their combination significantly extended animal survival. Our findings provide evidence that molecular glues targeting RBM39 and PARP inhibitors can be a promising combination therapy for ovarian HGSC.

Key words: RBM39, molecular glue, DCAF15, PARP1, ATM splicing regulation

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Mechanisms of switching COPI-mediated transport to autophagic degradation under starvation

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The selective removal of damaged or unnecessary proteins and organelles via autophagy is an essential for maintaining cellular homeostasis during starvation. COPI vesicles mediate transport between Golgi stacks and retrograde transport from the Golgi apparatus to the endoplasmic reticulum, with COPI-associated SCYL1 facilitates this trafficking. While COPI vesicles are implicated in autophagosome formation, the mechanisms underlying this contribution remain poorly understood. In this study, we used APEX2-based proximity labeling to identify proteins associated with membrane-conjugated LC3 in selective autophagy, revealing several candidates, including SCYL1. SCYL1 functions as a Golgiphagy receptor, mediating the autophagic degradation of Golgi components during starvation in a COPI-dependent manner. SCYL1 interacts with LC3 through an LIR motif to mediate COPI cargos for autophagic degradation, and defects in this motif leads to impaired Golgiphagy. Furthermore, SCYL1 enhances the recruitment of early autophagy-related proteins to COPI vesicles, promoting autophagosome formation. Notably, this process is inhibited by the phosphorylation of SCYL1 by the mTORC1 kinase. Under starvation conditions, mTORC1 inhibition reduces phosphorylation of SCYL1, which in turn enhances its interaction with LC3 and potentiates its role as a Golgiphagy receptor. Transcriptomic analyses and SCYL1 interactome profiling reveal significant alterations in multiple genes associated with membrane trafficking following SCYL1 knockdown. Taken together, our findings establish SCYL1 as a novel Golgiphagy receptor that facilitates COPI vesicle in mediating autophagosome formation under stress.

Key words: SCYL1, COPI vesicles, Golgiphagy.

Autophagic degradation of stress granules by the SQSTM1/p62-LC3A axis under high salt stress

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Stress granules (SGs), which form through liquid-liquid phase separation in response to cellular stress, are generally disassembled upon stress relief. However, under conditions of prolonged stress, persistent granules are generated, and these are often associated with neurodegenerative diseases. In this study, the selective autophagic degradation of SGs formed under high salt stress was investigated, with the LC3A isoform identified as a key mediator. Unlike the LC3B and LC3C isoforms, LC3A was observed to colocalize with high salt-induced SGs in a G3BP1/2-dependent manner. A proteomic analysis of affinity-purified SGs induced under high salt stress revealed enrichment of autophagy-related proteins, including LC3A, SQSTM1/p62, and VCP. The depletion of these proteins was shown to impair SG degradation significantly. Furthermore, SGs induced by high salt stress contained proteins involved in apoptosis, with their presence correlating with apoptosis suppression. The degradation of pathogenic inclusions formed by disease-associated variants, such as TDP-43Q331K or poly(GR) dipeptides from the C9orf72 hexanucleotide repeat expansion, was also found to be dependent on LC3A. These findings highlight the critical involvement of the SQSTM1-LC3A axis in the autophagic degradation of SGs during chronic stress and in pathological conditions, providing new insights on cellular stress responses and their implications for neurodegenerative diseases.

Key words: Stress granules, Selective autophagic degradation, SG degradation.

RanBP2 represses *interleukin-6* (*IL6*) gene expression by sumoylating and stabilizing ARGONAUTE 1 protein

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Ran-binding protein 2 (RanBP2), also known as Nucleoporin of 358 KDa (Nup358) is the main component of the cytoplasmic filaments of the nuclear pore complex. Four separate missense mutations in RanBP2 cause Acute Necrotizing Encephalopathy 1 (ANE1), which manifests as a sharp rise in cytokine production after common viral infections such as influenza and parainfluenza, which often leads to seizures, coma and high rate of mortality. However, how RanBP2 and its ANE1-associated mutations affect cytokine production is not well understood. Here we report that depletion of RanBP2 significantly increases the translation of *interleukin-6* (*IL-6*) mRNA, which encodes a cytokine that is aberrantly up-regulated in ANE1. Interestingly, the SUMO E3 ligase activity of RanBP2 and the let-7 miRNA binding site within the IL6 3'UTR are required for the repression of *IL6* mRNA translation, suggesting that sumoylation is required for efficient miRNA-based silencing. In support of this idea, we show that RanBP2-dependent sumoylation of argonaute protein prevents AGO1 degradation by reducing its ubiquitination, and that expression of AGO1 restores IL6-repession in cells that are defective in RanBP2-dependent sumoylation. Collectively, these results support a model whereby the RanBP2 SUMO E3 ligase activity promotes SUMOylation of AGO1, which in turn antagonizes the ubiquitination and subsequent degradation of AGO1 and ultimately enhances the miRNA-mediated suppression of mRNAs such as *IL6*.

Key words: RanBP2, sumoylation, ubiquitination, interleukin-6, Argonaute 1, protein degradation

Structural insights into the human HRD1 ubiquitin ligase complex

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In the endoplasmic reticulum (ER), defective proteins are cleaned via the ER-associated protein degradation pathway (ERAD). The HRD1 ubiquitin ligase complex with HRD1, SEL1L, XTP3B or OS9, and Derlin family proteins as the core components, plays essential roles in the recognition, retrotranslocation, and ubiquitination of luminal ERAD substrates. However, the molecular basis is unclear. Here, we determined the cryo-EM structure of the human HRD1-SEL1L-XTP3B complex at 3.3 Å resolution. HRD1 is a dimer, but only one protomer carries SEL1L-XTP3B complex, forming a 2:1:1 complex. Carefully checking the EM map reveals a trimmed N-glycan sandwiched by XTP3B and SEL1L, and SEL1L may also contribute to the recognition of trimmed glycan. The complex undergoes dramatic conformational changes when Derlin1 joins. The HRD1 dimer is broken, and two HRD1-SEL1L-XTP3B (1:1:1) units are joined together by a four-helix bundle forming by two SEL1L molecules. The four-helix bundle also touches the micelle, resulting in a bent transmembrane region. This indicates that Derlin1 engagement may lead to local curvature in the ER membrane. Cell-based functional assays were conducted to verify the structural observations. Our work represents a major step forward in the mechanistic elucidation of mammalian HRD1 complex-mediated ERAD.

Key words: ERAD, HRD1, structural biology

E2-based bioPROTAC as versatile modulators of intracellular protein targets

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Targeted protein degradation (TPD) through proteolysis-targeting chimeras (PROTACs) that hijack ubiquitin E3 ligases has emerged as a promising technology for therapeutic development and biological research. In this study, we identified eight ubiquitin E2 conjugating enzymes (UCEs) capable of efficiently degrading target proteins. Based on this discovery, we developed a novel targeted protein degradation technology, termed UCE-bioPROTAC, by directly fusing the target-binding sequence to the target substrate to the C-terminus of an E2 enzyme. The effectiveness of UCE-bioPROTAC was demonstrated by the successful degradation of a diverse range of neosubstrates, including PCNA, CDK2, MDM2, and BRD4. Importantly, our results show that UCE-bioPROTAC can be achieved through various delivery methods, such as DNA or mRNA transfection, or exocytotic delivery of recombinantly expressed proteins, further confirming its robustness and ease of use. Mechanistic studies have revealed that UCE-bioPROTAC mediates substrate degradation through both the proteasome and lysosome pathways. In conclusion, this study demonstrates the versatility of E2 enzymes as tools for promoting substrate protein degradation. Moreover, UCE-bioPROTAC expands the scope of current PROTAC technology, presenting new opportunities for targeted protein degradation.

Key words: Ubiquitin-proteasome system，UCE-Bioprotac, Protein degradation

Inactivation of GH3.5 by COP1-mediated K63-linked ubiquitination promotes seedling hypocotyl elongation

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CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1), which was first discovered as a central repressor of photomorphogenesis in *Arabidopsis*, destabilizes proteins by ubiquitination in both plants and animals. However, it is unclear whether and how *Arabidopsis* COP1 mediates non-proteolytic ubiquitination to regulate photomorphogenesis. Here, we showed that COP1-mediated lysine 63 (K63)-linked polyubiquitination inhibited the enzyme activity of GRETCHEN HAGEN 3.5 (GH3.5), a synthetase that conjugates amino acids to indole-3-acetic acid (IAA), thereby promoting hypocotyl elongation in the dark. We showed that COP1 physically interacted with and genetically acted through GH3.5 to promote hypocotyl elongation. COP1 had no effect on GH3.5 protein stability, but suppressed GH3.5 activity through K63-linked ubiquitination in the dark, inhibiting the endogenous conversion of IAA to IAA-amino acid conjugates. Further, light regulated IAA metabolism by suppressing the inhibitory effect of COP1 on the function of GH3.5 and its homologs. Our study identified an E3–substrate pair during K63 ubiquitination, which is largely unknown in plant biology. Our results also shed light on the non-proteolytic role of COP1-mediated ubiquitination and the mechanism by which light regulates auxin metabolism to modulate hypocotyl elongation.

Key words: Arabidopsis, K63-linked ubiquitination, COP1, auxin

USP7 Promotes Invasion and Metastasis of Intrahepatic Cholangiocarcinoma via Stabilizing NFKB2 and Activating Non-Canonical NF-κB Signaling

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Intrahepatic cholangiocarcinoma (ICC) is an aggressive form of liver cancer with limited treatment options and poor prognosis, primarily due to its high metastatic potential. While the ubiquitin-specific protease family has been implicated in various cancer-related processes, the specific role of USP7 in ICC progression and metastasis remains poorly understood. Understanding the molecular mechanisms underlying ICC metastasis is crucial for identifying new therapeutic targets, yet the involvement of USP7 in this context has not been fully explored.

Here, we demonstrate that USP7 expression is significantly upregulated in ICC tissues and cell lines, and its overexpression is strongly correlated with increased tumor invasiveness and worse patient outcomes. Functional assays revealed that USP7 promotes ICC cell migration and invasion by activating the non-canonical NF-κB signaling pathway. Mechanistically, USP7 stabilizes NFKB2 by preventing its ubiquitin-mediated degradation, leading to the accumulation of its active form, P52. This, in turn, drives the expression of downstream genes involved in invasion and metastasis. In vivo experiments further confirmed that USP7 overexpression enhances the metastatic potential of ICC cells.

Our findings establish the USP7/NFKB2 axis as a key driver of ICC metastasis through the activation of non-canonical NF-κB signaling. This highlights USP7 as a potential therapeutic target in ICC, offering new avenues for interventions aimed at curbing the metastatic spread of this highly lethal cancer.

Key words: Intrahepatic cholangiocarcinoma, USP7, Non-canonical NF-κB signaling, Ubiquitin-mediated degradation

A novel F-Box protein on the mitochondria involves in the development of the gastric cancer by inhibiting oxidative phosphorylation.

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This research project originated from our collaboration with Zhejiang Cancer Hospital. Through multi-omics analysis of clinical samples from over 100 patients, we identified a specific F-box protein, plays a crucial role in the onset and progression of gastric cancer. This discovery led us to investigate the mechanisms by which it influences gastric cancer development. We found that it has colocalization with mitochondria, a novel observation not previously reported in research articles or listed in UniProt. Proximity labeling experiments also revealed that it interacts with mitochondrial associated proteins. Then we use Co-immunoprecipitation (Co-IP) assays to testify the results of proximity labeling assays and further demonstrated that it binds to the channel proteins TOM20 and TOM40 on the mitochondrial outer membrane. Using structured illumination microscopy (SIM), we confirmed that it is situated within the outer mitochondrial membrane, which is atypical for an E3 ubiquitin ligase. The results indicate that this F-box protein appears to use TOM20 as a receptor to guide it to the mitochondria, ultimately entering the mitochondrial outer membrane through a channel formed by TOM40.

We then explored its functions in the mitochondria. Given that clinical sample data indicate this F-box protein has a negative regulatory effect on oxidative phosphorylation, we conducted ATP assays and Seahorse experiments, confirming that its overexpression can indeed downregulate ATP production. Additionally, we also observed that this prtoein may be involved in and responsive to mitophagy. Under CCCP-induced mitophagy conditions, it seems to undergo a modification and is subsequently recruited to the mitochondria, with its distribution in the mitochondria increasing as the degree of mitophagy deepens.

Key words: F-box, mitochondria, oxidative phosphorylation, mitophagy

Glucose-induced CRL4^{COP1}-p53 axis amplifies glycometabolism to drive tumorigenesis

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The diabetes-cancer association remains underexplained. Here, we describe a glucose-signaling axis that reinforces glucose uptake and glycolysis to consolidate the Warburg effect and overcome tumor suppression. Specifically, glucose-dependent CK2 O-GlcNAcylation impedes its phosphorylation of CSN2, a modification required for the deneddylase CSN to sequester Cullin RING ligase 4 (CRL4). Glucose, therefore, elicits CSN-CRL4 dissociation to assemble the CRL4^{COP1} E3 ligase, which targets p53 to derepress glycolytic enzymes. A genetic or pharmacologic disruption of the O-GlcNAc-CK2-CSN2-CRL4^{COP1} axis abrogates glucose-induced p53 degradation and cancer cell proliferation. Diet-induced overnutrition upregulates the CRL4^{COP1}-p53 axis to promote PyMT-induced mammary tumorigenesis in wild type but not in mammary-gland-specific p53 knockout mice. These effects of overnutrition are reversed by P28, an investigational peptide inhibitor of COP1-p53 interaction. Thus, glycometabolism self-amplifies via a glucose-induced post-translational modification cascade culminating in CRL4^{COP1}-mediated p53 degradation. Such mutation-independent p53 checkpoint bypass may represent the carcinogenic origin and targetable vulnerability of hyperglycemia-driven cancer.

Key words: Cullin RING ligase, Tumor suppressor p53, Warburg effect

IP6 toggles an intramolecular CSN2 switch to suppress hyperglycemia-driven Cullin neddylation and tumorigenesis

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Abstract

The Cullin RING ubiquitin ligases (CRLs) are activated by neddylation and regulated by dynamic complex formation with the deneddylase COP9 Signalosome (CSN). The metabolite inositol hexakisphosphate (IP_6) bridges CRL-CSN complexes. Whether and how IP_6 instills dynamics to the CRL-CSN system remains unclear. Here, we show that glucose deprivation increases the free pool of IP_6, in part by upregulating its synthase IP5K. Employing hydrogen/deuterium exchange coupled with mass spectrometry (HDX-MS), we further demonstrate that CSN2 exists in a closed form whereby its N-terminal acidic tail (NT) self-dock into the basic IP6-binding pocket. Glucose deprivation-induced IP6 competitively displaces CSN2-NT, bridging CRL-CSN while freeing the NT for CK2-mediated phosphorylation and avid docking into Cullin's basic canyon, thus forming high-affinity bidentate/two-pronged CSN2-CRL4 contact. Mice with mono-allelic CSN2-K70E mutation that abolishes IP6 binding are prone to tumor development due to hyperactive $CRL4^{COP1}$ and excessive p53 degradation, whereas IP6 treatment resulted in p53 stabilization and significant CRC regression. Thus, by transiting CSN2 from a closed to an extended conformation, the IP6 cofactor enables CSN inhibition of CRL in a glucose-regulated manner, consequently restraining the tumorigenic roles of the $CRL4^{COP1}$-p53 axis.

COP1 regulates fatty acid metabolism in liver

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Abstract

COP1 is a conserved E3 ubiquitin ligase, which senses light signals in plants to regulate circadian rhythms, and potentially senses nutrient signals in mammals. The key substrates are transcription factors such as HY5 (plants) or P53 (mammals). Lately, we found that the ubiquitination degradation of p53 by COP1 is strictly regulated by high glucose signal in cancer cells. However, p53 is not the main physiological substrate in COP1-hypomorphic mice. Liver is the core organ of nutrient storage and regulation in mammals. In order to find the key substrate of COP1 in normal tissues, we specifically knockout COP1 in the mouse liver ($COP1^{fl/fl}$:Alb-cre) and found that there was no difference between p53 and gluconeogenic transcription regulated by it, but the phenotype of fatty liver. When COP1 is deficient, triglyceride, lipid droplets and PLIN2 (lipid droplet stabilization) were accumulated in liver, while the transcription of cd36 (fatty acid uptake transport), dgat2 (lipogenesis) and pparγ (Regulation of fatty acid metabolism) was significantly upregulated, and the transcription of hsl (lipolysis) was significantly downregulated. The increased expression of C/EBPα and C/EBPβ-LIP may be the core transcription factors causing these phenomena, as they are also the key substrates of COP1. In addition, we found that both light and subsequently increased corticosterone upregulate the expression of COP1, suggesting that COP1 may also be a key E3 enzyme in the light-dark circadian rhythm in mammals, regulating the absorption and distribution of fatty acids and glucose during sleep.

Key word: COP1, C/EBPα, fatty liver，CD36

CSN2 plays a new role in regulating female thermogenesis via central nervous system

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Abstract

COP9 Signalosome Subunit 2(CSN2), a member of CSN complex, is able to bind to the Cullin Ring E3 Ligase (CRL) and inhibit CRL to ubiquitylate target proteins. At present, there are lots of studies on CSN in peripheral tissues and cancer, but few in the central nervous system (CNS). Here we found that CSN2 is involved in female thermogenesis via central nervous system. CSN2-K70E mutant mice, mimicking the CRL activation condition, has showed female obesity in early stage (less than 10-week-old), with more accumulation of white adipose tissue (WAT) and less thermogenesis from brown adipose tissue (BAT). According to thermoneutrality and BAT denervation experiments, there is no more difference between WT and mutant female mice, suggesting that CSN2 mediates energy metabolism from CNS. Our study reveals CSN2's new role in CNS control of peripheral thermogenesis, even controlling energy metabolism and obesity.

Key words: COP9 signalosome, CSN2, thermogenesis, central nervous system

Role of eIF3e in sarcomere formation and muscle development

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Skeletal muscle disorders represent a predominant cause of disability on a global scale. Genetic predispositions, neuropathies, and trauma-induced muscle atrophy significantly impair patient quality of life, with rehabilitation exercises currently constituting the principal therapeutic approach. Our investigation endeavors to elucidate the significance of mRNA translation and protein synthesis in the maintenance of skeletal muscle integrity, thereby unveiling potential avenues for enhancing muscle regeneration and addressing skeletal muscle pathology.

In the context of aging and muscle atrophy, an imbalance is observed between protein synthesis and degradation within muscle cells. Consequently, the investigation of mRNA spatial distribution and the modulation of protein synthesis by the translation initiation factor eIF3 within muscle cells is paramount for unraveling the etiology of muscle atrophy and fostering muscle regeneration.

In eIF3e-deficient heterozygous mice, alterations to the sarcomeric architecture were noted, specifically in the Z-disks and contractile elements. The ablation of eIF3e impedes the differentiation of myoblasts into myotubes, concomitant with a reduction in the expression of developmental proteins, including myosin heavy chain (MyHC), desmin, and myogenin (MyoG). eIF3e and eIF3f are observed to colocalize within the Z-disks, suggesting a potential role for the eIF3 complex in governing mRNA localization and local translation during sarcomere development. These findings highlight the indispensable role of eIF3e in skeletal muscle health and the preservation of sarcomere structure.

In summary, eIF3e, alongside possibly other eIF3 subunits, plays a pivotal role in the development, structural integrity, and functionality of striated muscles. Our research is thus dedicated to deciphering the precise function of eIF3e in maintaining sarcomeric structural and functional integrity, as well as in the process of muscle repair. This work lays the groundwork for the formulation of improved therapeutic strategies for muscular disorders.

Key words: eIF3 complex; muscle development;

The splicing factor SRRM2 modulates two S6K kinases to promote colorectal cancer growth

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Abstract

The mechanistic target of rapamycin (mTOR) pathway plays a critical role in cell growth and metabolic homeostasis. The ribosomal protein S6 kinases S6K1 and S6K2 are the major effectors of the mTOR pathway key to translation efficiency, but the mechanisms underlying their regulation remain largely unclear. In this study, we searched for mTOR regulators and found that the splicing factor SRRM2 modulates the levels of S6K1 and S6K2, thereby activating the mTOR-S6K pathway. Specifically, we demonstrated that SRRM2 facilitates the expression of S6K2 by modulating alternative splicing, and enhances the stability of the S6K1 protein by regulating the E3 ubiquitin ligase WWP2. Moreover, SRRM2 is highly expressed in colorectal cancer (CRC) tissues and is associated with a poor prognosis. SRRM2 promotes CRC growth in vitro and in vivo. Combined, these data reveal an oncogenic role of SRRM2 in CRC through activating the mTOR-S6K pathway by two different approaches, further suggesting SRRM2 as a potential therapeutic target for CRC.

UHRFl Facilitates Nonhomologous End-Joining Through K63-Linked Polyubiquitination of PAXX

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Abstract

DNA double-strand break (DSB) is an important inducer in tumorigenesis. Eukaryotes are equipped with several mechanisms to repair DSB, including non-homologous end joining (NHEJ) and homologous recombination (HR). PAXX is an important factor in the NHEJ repair pathway, and the PAXX protein is known to stabilize the Ku70/Ku80 heterodimer, promote KU accumulation, and maintain the stability of the core NHEJ complex. Here, we show that the E3 ubiquitin ligase UHRF1 is directly involved in the interaction between PAXX and KU. Under DNA damage conditions, UHRF1 has a direct interaction with PAXX, and UHRF1 mediates the K63-linked polyubiquitination of PAXX, promoting its recruitment to the damage site. Meanwhile, UHRF1 mediated ubiquitination of PAXX separates it from KU complex, which has positive effects on both genome stability and the efficiency of NHEJ repair. These findings illustrate the unique and critical functions of PAXX during NHEJ, highlighting its positive effects on both genome stability and the efficiency of NHEJ repair

Josephin Domain-Containing Protein 2 (JOSD2) Promotes Lung Cancer by Inhibiting LKB1 Activity

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Background: Non-small cell lung cancer (NSCLC) ranks as one of the leading causes of cancer-related deaths worldwide. Despite the prominence and effectiveness of kinase-target therapies in NSCLC treatment, these drugs are suitable for and beneficial to a mere ~30% of NSCLC patients. Consequently, the need for novel strategies addressing NSCLC remains pressing. Deubiquitinases (DUBs), a group of diverse enzymes with well-defined catalytic sites that are frequently overactivated in cancers and associated with tumorigenesis and regarded as promising therapeutic targets. Nevertheless, the mechanisms by which DUBs promote NSCLC remain poorly understood.

Methods: A comprehensive analysis of 97 DUBs using The Cancer Genome Atlas (TCGA) database was conducted to identify their impact on NSCLC patient survival. *In vitro* and *in vivo* experiments, including RNA interference and pharmacological inhibition, were performed to assess the effects of JOSD2 on NSCLC cell proliferation and LKB1 activity.

Results: High JOSD2 expression correlates with poor prognosis in NSCLC patients. JOSD2 depletion significantly inhibits NSCLC cell growth and disrupts tumor formation in xenograft models. Mechanistically, JOSD2 inhibits LKB1 kinase activity by removing K6-linked polyubiquitination, which is critical for maintaining the LKB1-STRAD-MO25 complex integrity. The first small-molecule inhibitor of JOSD2 was identified, demonstrating significant anti-tumor activity *in vitro* and *in vivo*.

Conclusion: JOSD2 plays a crucial oncogenic role in NSCLC by inhibiting LKB1 activity through deubiquitination. Targeting JOSD2 presents a promising therapeutic strategy for NSCLC, particularly in patients with inactivated LKB1.

Key words: Non-small cell lung cancer, Ubiquitination, Deubiquitinase (DUB), JOSD2, LKB1

USP10 Promotes Proliferation of Hepatocellular Carcinoma by Deubiquitinating and Stabilizing YAP/TAZ

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Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), are a pair of critical downstream effectors of the Hippo pathway that play important oncogenic roles in human cancers, particularly in hepatocellular carcinoma. However, both transcriptional coactivators YAP and TAZ are technically challenging to be directly targeted, which gives rise to the necessity to examine their posttranslational modifications (PTM) barcode. Here we focus on deubiquitinating enzymes (DUB), of which, the catalytic inhibition has been demonstrated to offer a novel strategy addressing the undruggability of their substrates. In this study, we globally profiled the contribution of DUB to both transcriptional activity and protein abundance of YAP/TAZ in hepatocellular carcinoma models and identified ubiquitin-specific peptidase 10 (USP10) as a potent YAP/TAZ-activating DUB. Mechanistically, USP10 can potently activate both YAP and TAZ by directly removing their polyubiquitin chains, a catalytic activity that stabilizes the protein levels of YAP and TAZ, and ultimately reinforces their oncogenic functions in hepatocellular carcinoma. Conversely, depletion of USP10 enhanced polyubiquitination of YAP/TAZ, promoted their proteasomal degradation, and ultimately arrested the proliferation of hepatocellular carcinoma in vitro and in vivo. Furthermore, the expression of USP10 positively correlates with the YAP/TAZ level in liver tumor tissues, and high USP10 level predicts poor prognosis in patients with hepatocellular carcinoma. Collectively, this study establishes the causal link between USP10 and hyperactivated YAP/TAZ in hepatocellular carcinoma cells identifies that USP10 would be a feasible therapeutic target for patients with hepatocellular carcinoma with prolonged activation of YAP/TAZ.

Key words: USP10; YAP/TAZ; hepatocellular carcinoma

Role of the eukaryotic translation initiation factor eIF3e in tumorigenesis

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An important hallmark of cancer cells is unrestrained cell growth, which needs abundant protein synthesis. Protein synthesis is a complex process accomplished by the ribosome and its associated factors. eIF3 is the largest but least understood protein synthesis associated factor and consists of 13 subunits. Overexpression of individual subunits can drive de novo holo-complex formation and modest increases in global protein synthesis along with cell transformation in vitro. However, eIF3e's proposed roles in cancer remain controversial, mostly due to lack of a molecular understanding of eIF3e function in vivo. Here, we are addressing eIF3e as a potential genetic modifier of cancer susceptibility in a p53 deficient mouse model of Li-Fraumeni syndrome. Tumor occurrence and growth rate will be measured to determine whether allelic reduction of eIF3e can protect the mice from tumorigenesis. Meanwhile, we have used CRISPR gene editing to delete single alleles of eIF3e or eIF3d in non-tumorigenic but p53 deficient MCF-10A cells. Remarkably, we found that dosis reduction of eIF3e or eIF3d genes does not affect the rate of cell proliferation. We are presently studying the impact on cell transformation by transducing these cells with mutant $HRAS^{G12V}$ oncogene. We are testing whether eIF3e/d reduction can prevent cell transformation in soft agar and in mice xenografts. Effects on mRNA selective translation are being studied by sucrose gradient centrifugation and ribosome profiling to obtain a molecular understanding of the function of eIF3 in cancer.

Key words: eIF3e reduction, tumorigenesis, Li-Fraumeni syndrome

SDHA/B reduction promotes hepatocellular carcinoma by facilitating the deNEDDylation of cullin1 and stabilizing YAP/TAZ

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Succinate dehydrogenase enzyme (SDH) is frequently diminished in Hepatocellular carcinoma (HCC) patient samples, and SDH reduction is associated with elevated succinate level and poor prognosis in HCC patients. But the underlying mechanisms about how impaired SDH activity promotes HCC remain unclear.

In this study, we observed remarkable downregulations of SDH subunits A and B (SDHA/B) in chronic liver injury-induced murine HCC models and patient samples. Subsequent RNA sequencing, hematoxylin & eosin (H&E) staining and immunohistochemistry (IHC) analyses of HCC samples revealed that Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) were significantly upregulated in HCC, with their levels inversely correlating with that of SDHA/B. YAP/TAZ stability was greatly enhanced in SDHA/B-depleted HCC cells along with accumulation of succinate. Further mechanistic analyses demonstrated that impaired activity of SDHA/B resulted in succinate accumulation which facilitated the deNEDDylation of cullin1, therefore disrupted the E3 ubiquitin ligase $SCF^{\beta\text{-}TrCP}$ complex, consequently led to YAP/TAZ stabilization and activation in HCC cells. The accelerated *in vitro* cell proliferation and *in vivo* tumor growth caused by SDHA/B reduction or succinate exposure were largely dependent on the aberrant activation of YAP/TAZ.

Our study demonstrated that SDHA/B reduction promotes HCC proliferation by preventing the proteasomal degradation of YAP/TAZ through modulating cullin1 NEDDylation, thus addicts SDH-deficient HCC cells to YAP/TAZ pathway and renders these cells vulnerable to YAP/TAZ inhibition. Our findings warrant further investigation on the therapeutic effects of targeting YAP/TAZ in HCC patients displaying reduced SDHA/B or elevated succinate levels.

Key words: Succinate dehydrogenase enzymes, Succinate, YAP/TAZ, HCC, deNEDDylation

Role of endothelial constitutive photomorphogenesis 9 (COP9) in atherosclerosis

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The constitutive photomorphogenesis 9 (COP9) signalosome (CSN) is a deNEDDylase controling the ubiquitination activity of cullin-RING-E3 ligases (CRLs) and thus the levels of various important cellular proteins. The CSN and its catalytic subunit CSN5 have been extensively studied in cancer, but its role in atherosclerosis is still poorly understood. Applying an atherogenic *Apoe*$^{-/-}$ mouse model of atherosclerosis, previous work from our lab had shown a role for myeloid Csn5, but the contribution of the endothelial CSN has remained unclear. Here we studied mice with an artery-endothelial-specific deletion of *Csn5* in high-fat diet (HFD)-fed hyperlipidemic *Apoe*$^{-/-}$ mice and performed mechanistic follow up experiments by siRNA pool-induced *Csn5* knock down in mouse aortic endothelial cells (MAECs). The CSN5 mimic MLN4924, which reduces CRL NEDDylation levels and activity, was also applied. Conditional deletion of *Csn5* in arterial endothelial cells in hyperlipidemic *Apoe*$^{-/-}$ mice (*Csn5*$^{\Delta arterial}$/*Apoe*$^{-/-}$) *in vivo* led to increased atherosclerotic plaque formation in aortic root and promoted plaque vulnerability. Of note, unbiased inflammatory array analysis identified tissue inhibitor of metalloproteinases (Timp-1), which was found to be decreased in plasma of *Csn5*$^{\Delta arterial}$/*Apoe*$^{-/-}$ mice, while MLN4924 treatment reduced plaque formation and upregulated Timp-1 levels. *In vitro*, *Csn5* knock-down in MAECs downregulated TIMP-1, but upregulated MMP2 and MMP9. Of note, bulk RNAseq analysis of *Csn5*-depleted versus control siRNA-treated MAECs indicated that *Csn5* silencing activates the Ifn-β pathway and enhanced interferon-induced protein IFI35 and the Ifn-driven inflammatory chemokine CXCL10. In conclusion, Arterial *Csn5* depletion promotes the size and vulnerability of atherosclerotic plaques by affecting the balance between TIMP-1 and MMPs and by upregulating a type I Ifn-driven inflammatory chemokine response.

Key words: COP9 signalosome, CSN5, atherosclerosis, endothelial cell, TIMP-1/MMPs, IFN-β/CXCL10

Characterizing the regulation of translation initiation factor eIF3k during the chronic ER stress response

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The integrated stress response (ISR) is a crucial adaptive pathway employed by eukaryotic cells to restore cellular homeostasis under diverse stress stimuli. Central to this pathway is the phosphorylation of eukaryotic translation initiation factor 2 alpha (eIF2α) by members of the eIF2α kinase family. While the role of eIF2α in stress response is well-established, the contribution of other translation factors such as eIF3k and eIF3l remains relatively unexplored. Recent studies have begun to shed light on the involvement of eIF3k and eIF3l in stress response and its potential implications in cellular and organismal processes.

It was shown that C. elegans strains deleted of eIF3k or eIF3l exhibit marked resistance to ER stress. Likewise, we have reported that eIF3k exhibits selective mRNA binding and its depletion promotes global translation, cell proliferation, tumor growth, and stress resistance. In addition, eIF3k is selectively downregulated during endoplasmic reticulum (ER) stress. Further investigation into the precise mechanisms underlying eIF3k regulation during stress response is warranted to advance our understanding of this complex pathway. Specifically, we are addressing the mechanism of ER stress-induced downregulation of eIF3k and eIF3l. We have already found that downregulation occurs through proteasome-dependent proteolysis. Our studies now focus on identifying the specific signals and enzymes targeting eIF3k and eIF3l for proteasomal degradation.

Key words: endoplasmic reticulum stress, eIF3k/l subunits, proteasomal degradation

Lipid-anchored Proteasomes Control Membrane Protein Homeostasis

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Protein degradation in eukaryotic cells is mainly carried out by the 26S proteasome, a macromolecular complex not only present in the cytosol and nucleus but also associated with various membranes. How proteasomes are anchored to the membrane and the biological meaning thereof have been largely unknown in higher organisms. Here we show that N-myristoylation of the Rpt2 subunit is a general mechanism for proteasome-membrane interaction. Loss of this modification in the Rpt2-G2A mutant cells leads to profound changes in the membrane-associated proteome, perturbs the endomembrane system and undermines critical cellular processes such as cell adhesion, endoplasmic reticulum-associated degradation (ERAD) and membrane protein trafficking. Rpt2G2A/G2A homozygous mutation is embryonic lethal in mice and is sufficient to abolish tumor growth in a nude mice xenograft model. These findings have defined an evolutionarily conserved mechanism for maintaining membrane protein homeostasis and underscored the significance of compartmentalized protein degradation by myristoyl-anchored proteasomes (MAPs) in health and disease.

Key words: 26S proteasome, Rpt2, myristoylation, membrane

Role of translation initiation factor eIF3 in hepatic metabolism and liver disease

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Eukaryotic initiation factor 3 (eIF3) plays a crucial role in protein synthesis by facilitating translation initiation. More recently, eIF3 has also been implicated in early translation elongation, especially of mRNAs encoding mitochondrial proteins. The liver acts as a central hub of protein synthesis, producing proteins essential for detoxification, immune response, nutrient metabolism, and energy homeostasis. In addition, GWAS studies have linked single nucleotide variants (SNVs) in several eIF3 subunits – in particular eIF3d and eIF3e – in liver disease. We therefore hypothesize that dysfunction of eIF3 subunits in the liver may result in metabolic disorders, in particular nonalcoholic fatty liver disease (NAFLD).

In preliminary studies, we have discovered that liver-specific knockout of eIF3a or eIF3e in mice is tolerated, but results in lipid accumulation as well as alterations in NAFLD-related blood markers. In our present studies, we are exploring the possibility that this phenotype is caused by a cell autonomous defect in hepatic mitochondrial activity. To uncover molecular mechanisms, we use ribosome profiling of mouse livers as well as primary mouse hepatocytes to assess transcript selective changes in the liver translatome.

Our findings indicate that the eIF3a and eIF3e subunits of eIF3 play a critical role in maintaining liver metabolic homeostasis. Further research is underway to fully understand the molecular mechanisms involved and to explore potential therapeutic targets.

Key words: eIF3a, eIF3e, protein synthesis, liver metabolism, NAFLD

Identification of potato SUMOylation pathway members and StSIZ1 interaction protein screening

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Among the post-translational modifications, SUMOylation is one of the crucial pathways that play an important role in protein stability alteration, subcellular localization, and interactions between proteins. Like ubiquitination, SUMOylation is an ATP-dependent enzymatic cascade reaction. The *SIZ1* gene belongs to the family of SUMO E3 ligases. *SIZ1* regulates SUMOylation in plants, thereby influencing physiological functions and stress such as drought, salinity and heat, by affecting the binding of various transcription factors and proteins to SUMO. The potato has a shallow root system and is susceptible to numerous abiotic stresses, especially water deficit conditions. Unravelling the functional mechanism of potato *SIZ1* is key to ensuring yield and quality. In this present study, we performed high-throughput screening and identification of SUMOylation pathway members in potatoes and StSIZ1 interaction protein screening. The main results obtained are as follows.

1. A local Hidden Markov Model (HMM) was constructed to retrieve members of the SUMOylation pathway in the whole potato genome and analyze the conserved structural domains one by one. Finally, seven SUMO, three SAE (E1), nine SCE (E2), and a single copy of SUMO ligase (E3) genes were identified. Chromosomal localization revealed that putative members of the SUMO pathway were localized on nine different chromosomes; the exon number ranged from two to thirteen.
2. Tissue expression specificity analysis of potato using qRT-PCR showed that StSIZ1 expression varied in stem, root, leaf and tuber. The results showed that the expression of StSIZ1 is significantly higher than that of the control group under PEG/NaCl stress and that the expression of StSIZ1 shows an increasing trend under different treatment periods (0 h, 3 h, 6 h, 12 h, 24 h) of these two stresses. The results indicate that StSIZ1 is involved in regulating drought tolerance in potato, affecting ABA and GA3 signaling regulated plant growth.
3. Subcellular localization pinpoints where StSIZ1 exerts its function. The yeast two-hybrid technique was used to screen the cDNA library of potato for StSIZ1 interacting proteins, and 23 interacting proteins were identified, including CBL-interacting protein kinase, NAC domain-containing protein, basic leucine zipper and protein phosphatase 2A-4.

Key words: potato, SUMOylation pathway, *StSIZ1*, drought stress,

Molecular basis for C-degron recognition by CRL2^{APPBP2} ubiquitin ligase

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E3 ubiquitin ligases determine the specificity of eukaryotic protein degradation by selective binding to destabilizing protein motifs, termed degrons, in substrates for ubiquitin-mediated proteolysis. The exposed C-terminal residues of proteins can act as C-degrons that are recognized by distinct substrate receptors (SRs) as part of dedicated cullin-RING E3 ubiquitin ligase (CRL) complexes. APPBP2, an SR of Cullin 2-RING ligase (CRL2), has been shown to recognize R-x-x-G/C-degron; however, the molecular mechanism of recognition remains elusive. By solving several cryogenic electron microscopy structures of active CRL2^{APPBP2} bound with different R-x-x-G/C-degrons, we unveiled the molecular mechanisms underlying the assembly of the CRL2^{APPBP2} dimer and tetramer, as well as C-degron recognition. The structural study, complemented by binding experiments and cell-based assays, demonstrates that APPBP2 specifically recognizes the R-x-x-G/C-degron via a bipartite mechanism; arginine and glycine, which play critical roles in C-degron recognition, accommodate distinct pockets that are spaced by two residues. In addition, the binding pocket is deep enough to enable the interaction of APPBP2 with the motif placed at or up to three residues upstream of the C-end. Overall, our study not only provides structural insight into CRL2^{APPBP2}-mediated protein turnover but also serves as the basis for future structure-based chemical probe design.

A novel quality control pathway on the ribosome dedicated for co-translational protein complex-assembly

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At the critical junction of translation and protein folding, the ribosome serves as a central hub, orchestrating the actions of various factors that facilitate the maturation of emerging polypeptide-chains. These include modifying enzymes, targeting factors, and folding chaperones. Recently, it was discovered that the assembly of higher-order oligomeric complexes, the final stage of folding, also occurs during protein synthesis. Moreover, subunits prone to misfolding are protected from aggregation and degradation through co-translational interactions with their partner subunits. This raises the question: How does the cell detect protein misfolding before translation is complete? To investigate this, we targeted ribosomes synthesizing misfolding-prone complex subunits and analysed their interactome. Proteomics analysis led to the discovery of a novel quality-control pathway dedicated for misassembled protein subunits. This pathway can recognize nascent-chains exposing unassembled interface harboring domains, already during translation. In this pathway, we identified several previously uncharacterized proteins, which we termed NQC1,2,3 (Nascent-chain Quality Control). This pathway is coordinated with the ribosome-associated chaperone Chp1 (CHaPerone), previously identified as dedicated to the synthesis of eEF1A (eukaryotic translation Elongation Factor 1 Alpha) as well as mRNA localization factors. Ribosome-directed N`-degradomics analysis of strains lacking NQC1-3, compared to CHP1, and Ribosome Quality-Control E3 ubiquitin ligase LTN1 (Listerin1) deletion strains, revealed that under translation stress, Nqc1-3 and Chp1 are essential for protecting the nascent proteome. Deletion strains exhibited reduced degradation of nascent-chains, with specialized substrate pools compared to *ltn1Δ*. Deletion strains furthermore exhibited impaired growth rates under translation stress, similar to *ltn1Δ*. Double deletion of these novel factors with *LTN1* resulted in an even more severe reduction in growth rates, suggesting they function in parallel pathways. This research provides proteome-wide insights into factors that safeguard the cellular proteome during synthesis. It can lead to novel therapeutic strategies for diseases linked to protein misfolding, such as Parkinson’s and Alzheimer’s.

Key words: 26S proteasome, Ribosome, Co-translational complex assembly, Chaperoning

PTEN neddylation aggravates CDK4/6 inhibitor resistance in breast cancer

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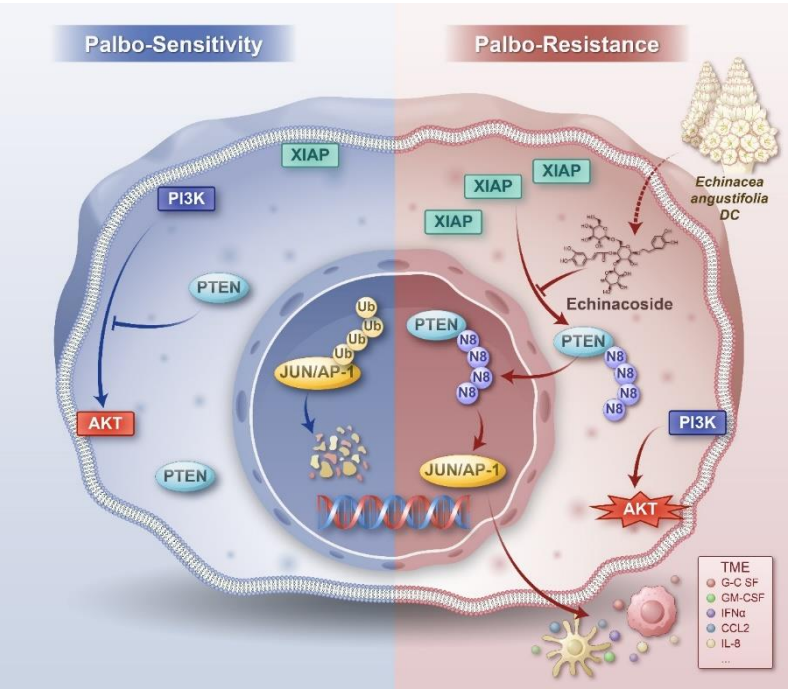
ABSTRACT

The gradual emergence of a novel therapeutic approach lies in the restoration of tumor suppressive machinery. PTEN, a renowned tumor suppressor, plays a pivotal role in oncogenesis, particularly through its post-translational modifications. PTEN neddylation contributes to its inactivation and fuels breast cancer progression. Here, our research highlights the critical role of PTEN neddylation in the development of resistance to Palbociclib, a CDK4/6 inhibitor commonly used in HR+/HER2- breast cancer patients. Notably, elevated level of neddylated PTEN is markedly associated with Palbociclib resistance. Strikingly, we identified a natural and potent inhibitor of PTEN neddylation which could obviously re-sensitized Palbociclib-therapy-resistant breast cancer *in vitro* and *in vivo*. Mechanistically, PTEN neddylation initiates activation of the PI3K/Akt signaling pathway, whereas nuclear PTEN neddylation contributes to the stabilization of JUND by disrupting the delicate interplay between JUND and the E3 ubiquitin ligase, ITCH. Subsequently, this stabilization serves to amplify the activation of the AP-1/MAPK signaling pathway, resulting in the release of G-CSF, GM-CSF, IFN-γ, IL-1RA, IL-8. These cytokines and chemokines, in turn, reprogram the tumor microenvironment, contributing to drug resistance, tumor recurrence and metastasis. This novel insight underscores the potential of targeting PTEN neddylation as a promising strategy for restoring the activity of key tumor suppressor and overcoming resistance in breast cancer therapy.



Function of BbCUL3 in *Beauveria bassiana*

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Abstract

Beauveria bassiana is an entomopathogenic fungus with a wide spectrum of insect hosts, which plays an important role in biological control of agricultural and forestry pests.CUL3, as the central scaffold of CUL3-RING E3 ubiquitin ligase, recruits specific protein substrates through BTB protein, and participates in the regulation of many important biological processes by regulating ubiquitination of substrates. However, what role does BbCUL3 play in *Beauveria bassiana*?In order to answer this question, we use homologous recombination principle to knock out *BbCul3*.The results showed that the the growth and development of *ΔBbcul3* was abnormal, which showed the fluffy phenotype of colonies, abnormal morphology of terminal hyphae, early germination and decreased sporulation. The pigment synthesis of *ΔBbcul3* also decreased significantly. Surprisingly, the virulence of *ΔBbcul3* is not much different from that of WT, but the ability to penetrate insect carcasses is significantly weakened.In addition, we found a BTB protein with kelch domain that interacts stably with BbCUL3 through yeast double hybrid, and named it BbKLHL1.The germination of *ΔBbklhl1* was also advanced; At the same time, it was found that the virulence of *ΔBbklhl1* was enhanced, which explained that the virulence of *ΔBbcul3* was not weakened when the sporulation was significantly reduced.Finally, these results show the importance of BbCUL3 to *Beauveria bassiana*, especially in the regulation of sporulation and virulence, which provides a direction and lays a foundation for the later mechanism analysis.

CCT7 functions as a monosome for the clearance of ALS-related aggregates

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ALS is a devastating motor neuron disease that leads to progressive muscle weakness and paralysis, with no current cure. CCT complex is a type II chaperone protein in archaea and all eukaryotic cells, which maintains protein homeostasis. It may affect the onset and progression of ALS, but the specific molecular mechanisms are unclear. Recent studies have shown that CCT subunit CCT2 acts as a monomer to clear aggregated proteins through autophagy, which is called aggrephagy. Our investigations also found that CCT7 could form an aggresome-like structure with its monomer status, which enriched ALS-related proteins. Besides, the aggresome-like structure was partially colocalized with LC3B, an autophagy marker. Additionally, CCT7 is expressed in motor neurons and partially co-localizes with the ALS-associated protein SOD1 in the spinal cord of ALS mice. Based on these findings, It is speculated that CCT7 may regulate the expression of ALS-related proteins through phagocytosis different from other subunits of CCT, thus influencing the onset and progression of ALS. These findings provide potential novel targets for understanding and treating ALS, offering new insights for ALS therapy.

Keywords: Chaperonin, TRiC/CCT, Aggrephagy, ALS

USP43 Regulating K33 Ubiquitination of STAT1 Protein controls antiviral interferon signaling

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The interferon (IFN) has a broad spectrum of antiviral function. In clinical diagnosis and treatment, the application of IFN helps patients with chronic hepatitis B (CHB) achieve clinical cure, but only a small part of this population accounts for less than 12%. Therefore, it is of great significance to explore the regulation mechanism and influencing factors of IFN antiviral function in order to improve the functional cure rate of CHB patients. The ubiquitin-proteasome system (UPS), plays a key role in cell metabolism, biological signal transduction, gene expression regulation and other aspects. Among them, deubiquitinating enzymes (DUBs) can affect the stability, localization, function and interaction of proteins by removing ubiquitin molecules from substrates. Our study found that ubiquitin specific peptidase 43 (USP43) regulates the IFN signaling pathway, thereby inhibiting the antiviral effect of IFN. We found that USP43 regulates STAT1-K33-related ubiquitination, which can be regulated by RNF2 for the first time. By removing the K48-linked ubiquitination of RNF2, it can stabilize RNF2 and promote the K33-linked ubiquitination. After STAT1 is ubiquitinated at K33-linked, it can inhibit the level of p-STAT1, down-regulate the transcriptional activity of ISRE and the expression of ISGs, and ultimately negatively regulate the antiviral function of IFN.

Key words: Ub-Proteasome, IFN, signal transduction, USP43, RNF2

Distinct amino acid-based PROTACs target the oncogenic kinases for degradation in non-small cell lung cancer (NSCLC)

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Abstract:

Proteolysis-targeting chimeras (PROTACs) selectively eliminate detrimental proteins by exploiting the ubiquitin-proteasome system (UPS), representing a promising therapeutic strategy against various diseases. Effective adaptations of degradation signal sequences and E3 ligases for PROTACs remain limited. Here, we employed three amino acids - Gly, Pro, and Lys - as the ligand to recruit the corresponding E3 ligases: CRL2ZYG11B/ZER1, GID4, and UBRs, to degrade EML4-ALK and mutant EGFR, two oncogenic drivers in NSCLC. We found that the extent of EML4-ALK and EGFR reduction can be easily fine-tuned by using different degradation signals. These amino acid-based PROTACs, termed AATacs, hindered proliferation and induced cell cycle arrest and apoptosis of NSCLC cells in vitro. Compared to other PROTACs, AATacs are small, interchangeable but with different degradation efficiency. Our study further expands the repertoire of E3 ligases and their ligands for PROTAC application, improving the versatility and utility of targeted protein degradation for therapeutic purposes.

STUB1-mediated Ubiquitination and Degradation of NSUN2 Promotes Hepatocyte Ferroptosis by Decreasing m^5C Methylation of *Gpx4* mRNA

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Ferroptosis is an iron-dependent cell death that occurs due to the peroxidation of phospholipids in the cell membrane. In this study, we find that the protein levels of NSUN2, one of the most important RNA methyltransferases, is significantly decreased in hepatocyte ferroptosis. STIP1 homology and U-box protein 1 (STUB1), a crucial member of the RING family E3 ubiquitin ligase, mediates the ubiquitination and promotes the degradation of NSUN2 in ferroptosis, with lysines 457 and 654 in NSUN2 as key sites. NSUN2 ubiquitination level is decreased by knockdown of STUB1, while rescue of STUB1 expression in STUB1-silenced cells could restore NSUN2 ubiquitination level. Furthermore, it is found that NSUN2 binds with STUB1 and Hsp70, which indicates that Hsp70 is a co-factor for the ubiquitination of NSUN2 by STUB1 in ferroptosis. NSUN2 ubiquitination may contain both K48- and K63-linked chains, with K63-linked ubiquitin chains being the main form. Selenoprotein glutathione peroxidase 4 (GPX4) is a prominent suppressor of ferroptosis. It is found that downregulation of NSUN2 diminishes m^5C methylation of *Gpx4* mRNA 3'UTR. The reduction of NSUN2-mediated *Gpx4* mRNA m^5C methylation abrogates the interaction between SBP2 and the selenocysteine insertion sequence (SECIS), and leads to inhibition of GPX4 protein expression. Lower GPX4 expression promotes hepatocyte ferroptosis *in vivo* and *in vitro*, which is reversed by restoration of NSUN2. These findings shed light on the mechanism of NSUN2 modification and degradation, and also indicate that the STUB1-NSUN2-GPX4 axis plays a regulatory role in hepatocyte ferroptosis.

Key words: NSUN2; Ferroptosis; STUB1; ubiquitination; GPX4; m^5C methylation

Ufd2 regulates DNA negative supercoils and crossover interference during meiosis

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Abstract

Ubiquitination plays an essential role in sexual reproduction and gametogenesis. However, ubiquitin E3 ligases and their substrates in these processes remains less known. Here, we show that E3/E4 ubiquitin ligase Ufd2p locates on meiotic recombination sites and is required for meiotic recombination in budding yeast. Further studies show that Ufd2p mediates the ubiquitination of Topoisomerase II (Top2p) and maintains levels of DNA negative supercoil, and thus regulates the strength of crossover interference and numbers of meiotic recombination. Together, our findings identify a novel factor Ufd2p in promoting meiotic recombination and uncover the detail molecular mechanisms.

Key words: Ufd2p, Meiosis, Meiotic recombination, Topoisomerase II, Crossover interference

Peptide-based PROTAC degrader of HMGB1 suppresses colorectal cancer and modulates tumor microenvironment

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Colorectal cancer (CRC) is among the most prevalent and deadly cancers globally, with the third highest incidence and the fifth highest mortality rate in China. Surgical resection remains the primary treatment, while targeted therapy and immunotherapy are deployed for advanced cases. However, these treatments face challenges such as off-target effects, significant side effects, and the potential for drug resistance. The development of efficient, low-toxicity drugs that are less likely to induce resistance is therefore of great market potential. The overexpression of High Mobility Group Box 1 (HMGB1) protein is associated with poor prognosis and increased metastasis in cancer. HMGB1 promotes the proliferation, migration, and invasion of tumor cells through various signaling pathways and also impacts immune cells within the tumor microenvironment. PROTAC technology is an innovative method for protein degradation that forms complexes to guide the degradation of target proteins, which has been applied to the treatment of various diseases, including cancer. Compared to traditional small molecule drugs, PROTAC can degrade the entire protein, avoiding compensatory protein expression and reducing the likelihood of tumor drug resistance. This paper utilizes phage display technology to screen for peptides with high affinity for HMGB1, constructs PROTAC molecules targeting HMGB1, and verifies the degradation capacity and anti-tumor activity of HMGB1-PROTAC through a variety of experimental models, including cells, organoids, and animal models. The inhibitory effects of HMGB1-PROTAC on the proliferation and invasive metastasis capabilities of colorectal and liver cancer cells are validated. The research also explores the role and mechanism of HMGB1-PROTAC in the tumor immune microenvironment. The research presented in this paper contributes to the growing body of knowledge on PROTAC technology and its application in cancer treatment, particularly in the context of colorectal and liver cancers.

Key words: PROTAC, Colorectal cancer, Tumor immune microenvironment, HMGB1,