Lit Lunch 12-12-12

JC 13 dec 2013

Plant Journal

Versatile roles of Arabidopsis plastid ribosomal proteins in plant growth and development

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Summary

A lack of individual plastid ribosomal proteins (PRPs) can have diverse phenotypic effects in *Arabidopsis thaliana*, ranging from embryo lethality to compromised vitality, with the latter being associated with photosynthetic lesions and decreases in the expression of plastid proteins. In this study, reverse genetics was employed to study the function of eight PRPs, five of which (PRPS1, -S20, -L27, -L28 and -L35) have not been functionally characterised before. In the case of PRPS17, only leaky alleles or RNA interference lines had been analysed previously. PRPL1 and PRPL4 have been described as essential for embryo development, but their mutant phenotypes are analysed in detail here. We found that PRPS20, -L1, -L4, -L27 and -L35 are required for basal ribosome activity, which becomes crucial at the globular stage and during the transition from the globular to the heart stage of embryogenesis. Thus, lack of any of these PRPs leads to alterations in cell division patterns, and embryo development ceases prior to the heart stage. PRPL28 is essential at the latest stages of embryo–seedling development, during the greening process. PRPS1, -S17 and -L24 appear not to be required for basal ribosome activity and the organism can complete its entire life cycle in their absence. Interestingly, despite the prokaryotic origin of plastids, the significance of individual PRPs for plant development cannot be predicted from the relative phenotypic severity of the corresponding mutants in prokaryotic systems.

Plant Journal

The combined use of photoaffinity labeling and surface plasmon resonance-based technology identifies multiple salicylic acid-binding proteins

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Summary

Salicylic acid (SA) is a small phenolic molecule that not only is the active ingredient in the multi-functional drug aspirin, but also serves as a plant hormone that affects diverse processes during growth, development, responses to abiotic stresses and disease resistance. Although a number of SA-binding proteins (SABPs) have been identified, the underlying mechanisms of action of SA remain largely unknown. Efforts to identify additional SA targets, and thereby elucidate the complex SA signaling network in plants, have been hindered by the lack of effective approaches.

Here, we report two sensitive approaches that utilize SA analogs in conjunction with either a photoaffinity labeling technique or surface plasmon resonance-based technology to identify and evaluate candidate SABPs from Arabidopsis. Using these approaches, multiple proteins, including the E2 subunit of α-ketoglutarate dehydrogenase and the glutathione *S*-transferases GSTF2, GSTF8, GSTF10 and GSTF11, were identified as SABPs. Their association with SA was further substantiated by the ability of SA to inhibit their enzymatic activity. The photoaffinity labeling and surface plasmon resonance-based approaches appear to be more sensitive than the traditional approach for identifying plant SABPs using size-exclusion chromatography with radiolabeled SA, as these proteins exhibited little to no SA-binding activity in such an assay. The development of these approaches therefore complements conventional techniques and helps dissect the SA signaling network in plants, and may also help elucidate the mechanisms through which SA acts as a multi-functional drug in mammalian systems.

Plant Journal

Differential remodeling of the lipidome during cold acclimation in natural accessions of *Arabidopsis thaliana*

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Summary

Freezing injury is a major factor limiting the geographical distribution of plant species and the growth and yield of crop plants. Plants from temperate climates are able to increase their freezing tolerance during exposure to low but non-freezing temperatures in a process termed cold acclimation. Damage to cellular membranes is the major cause of freezing injury in plants, and membrane lipid composition is strongly modified during cold acclimation. Forward and reverse genetic approaches have been used to probe the role of specific lipid-modifying enzymes in the freezing tolerance of plants. In the present paper we describe an alternative ecological genomics approach that relies on the natural genetic variation within a species. *Arabidopsis thaliana* has a wide geographical range throughout the Northern Hemisphere with significant natural variation in freezing tolerance that was used for a comparative analysis of the lipidomes of 15 Arabidopsis accessions using ultra-performance liquid chromatography coupled to Fourier-transform mass spectrometry, allowing the detection of 180 lipid species. After 14 days of cold acclimation at 4°C the plants from most accessions had accumulated massive amounts of storage lipids, with most of the changes in long-chain unsaturated triacylglycerides, while the total amount of membrane lipids was only slightly changed. Nevertheless, major changes in the relative amounts of different membrane lipids were also evident. The relative abundance of several lipid species was highly correlated with the freezing tolerance of the accessions, allowing the identification of possible marker lipids for plant freezing tolerance.

Plant Journal

Studies of *Physcomitrella patens* reveal that ethylene-mediated submergence responses arose relatively early in land-plant evolution

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Summary

Colonization of the land by multicellular green plants was a fundamental step in the evolution of life on earth. Land plants evolved from fresh-water aquatic algae, and the transition to a terrestrial environment required the acquisition of developmental plasticity appropriate to the conditions of water availability, ranging from drought to flood. Here we show that extant bryophytes exhibit submergence-induced developmental plasticity, suggesting that submergence responses evolved relatively early in the evolution of land plants. We also show that a major component of the bryophyte submergence response is controlled by the phytohormone ethylene, using a perception mechanism that has subsequently been conserved throughout the evolution of land plants. Thus a plant environmental response mechanism with major ecological and agricultural importance probably had its origins in the very earliest stages of the colonization of the land.

JBC

The Organization of a CSN5-containing Subcomplex of the COP9 Signalosome

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Abstract

The COP9 signalosome (CSN) is an evolutionarily conserved multi-protein complex that interfaces with the ubiquitin-proteasome pathway and plays critical developmental roles in both animals and plants. Although some subunits are present only in an ~320-kDa complex-dependent form, other subunits are also detected in configurations distinct from the 8-subunit holocomplex. To date, the only known biochemical activity intrinsic to the complex, deneddylation of the Cullin subunits from Cullin-RING ubiquitin ligases, is assigned to CSN5. As an essential step to understanding the structure and assembly of a CSN5-containing subcomplex of the CSN, we reconstituted a CSN4-5-6-7 subcomplex. The core of the subcomplex is based on a stable heterotrimeric association of CSN7, CSN4, and CSN6, requiring coexpression in a bacterial reconstitution system. To this heterotrimer, we could then add CSN5 *in vitro* to reconstitute a quaternary complex. Using biochemical and biophysical methods, we identified pairwise and combinatorial interactions necessary for the formation of the CSN4-5-6-7 subcomplex. The subcomplex is stabilized by three types of interactions: MPN-MPN between CSN5 and CSN6, PCI-PCI between CSN4 and CSN7, and interactions mediated through the CSN6 C terminus with CSN4 and CSN7. CSN8 was also found to interact with the CSN4-6-7 core. These data provide a strong framework for

further investigation of the organization and assembly of this pivotal regulatory complex.

Journal of Molecular Biology

Impaired Folding of the Mitochondrial Small TIM Chaperones Induces Clearance by the i-AAA Protease

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The intermembrane space of mitochondria contains a dedicated chaperone network—the small translocase of the inner membrane (TIM) family—for the sorting of hydrophobic precursors. All small TIMs are defined by the presence of a twin CX_3C motif and the monomeric proteins are stabilized by two intramolecular disulfide bonds formed between the cysteines of these motifs. The conserved cysteine residues within small TIM members have also been shown to participate in early biogenesis events, with the most N-terminal cysteine residue important for import and retention within the intermembrane space via the receptor and disulfide oxidase, Mia40. In this study, we have analyzed the in vivo consequences of improper folding of small TIM chaperones by generating site-specific cysteine mutants and assessed the fate of the incompletely oxidized proteins within mitochondria. We show that no individual cysteine residue is required for the function of Tim9 or Tim10 in yeast and that defective assembly of the small TIMs induces their proteolytic clearance from mitochondria. We delineate a clearance mechanism for the mutant proteins and their unassembled wild-type partner protein by the mitochondrial ATP-dependent protease, Yme1 (yeast mitochondrial escape 1).

Structural Insights on the Plant Salt-Overly-Sensitive 1 (SOS1) Na^+/H^+ Antiporter

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The Arabidopsisthaliana Na^+/H^+ antiporter salt-overly-sensitive 1 (SOS1) is essential to maintain low intracellular levels of toxic Na^+ under salt stress. Available data show that the plant SOS2 protein kinase and its interacting activator, the SOS3 calcium-binding protein, function together in decoding calcium signals elicited by salt stress and regulating the phosphorylation state and the activity of SOS1. Molecular genetic studies have shown that the activation implies a domain reorganization of the antiporter cytosolic moiety, indicating that there is a clear relationship between function and molecular structure of the antiporter. To provide information on this issue, we have carried out in vivo and in vitro studies on the oligomerization state of SOS1. In addition, we have performed electron microscopy and single-particle reconstruction of negatively stained full-length and active SOS1. Our studies show that the protein is a homodimer that contains a membrane domain similar to that found in other antiporters of the family and an elongated, large, and structured cytosolic domain. Both the transmembrane (TM) and cytosolic moieties contribute to the dimerization of the antiporter. The close contacts between the TM and the cytosolic domains provide a link between regulation and transport activity of the antiporter.

A Nitrogen-Regulated Glutamine Amidotransferase (GAT1_2.1) Represses Shoot Branching in Arabidopsis

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Shoot branching in plants is regulated by many environmental cues and by specific hormones such as strigolactone (SL). We show that the GAT1_2.1 gene (At1g15040) is repressed over 50-fold by nitrogen stress, and is also involved in branching control. At1g15040 is predicted to encode a class I glutamine amidotransferase (GAT1), a superfamily for which Arabidopsis (Arabidopsis thaliana) has 30 potential members. Most members can be categorized into known biosynthetic pathways, for the amidation of known acceptor molecules (e.g. CTP synthesis). Some members, like GAT1_2.1, are of unknown function, likely involved in amidation of unknown acceptors. A gat1_2.1 mutant exhibits a significant increase in shoot branching, similar to mutants in SL biosynthesis. The results suggest that GAT1_2.1 is not involved in SL biosynthesis since exogenously applied GR24 (a synthetic SL) does not correct the mutant phenotype. The subfamily of GATs (GATase1_2), with At1g15040 as the founding member, appears to be present in all plants (including mosses), but not other organisms. This suggests a plant-specific function such as branching control. We discuss the possibility that the GAT1_2.1 enzyme may activate SLs (e.g. GR24) by amidation, or more likely could embody a new pathway

for repression of branching.

QUANTITATIVE PROTEOMICS REVEAL FACTORS REGULATING RNA BIOLOGY AS DYNAMIC TARGETS OF STRESS-INDUCED SUMOYLATION IN *ARABIDOPSIS*

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MCP Papers in Press. Published on November 29, 2012 as Manuscript M112.025056

The stress-induced attachment of small ubiquitin-like modifier (SUMO) to a diverse collection of nuclear proteins regulating chromatin architecture, transcription, and RNA biology has been implicated in protecting plants and animals against numerous environmental challenges. To better understand stress-induced SUMOylation, we combined stringent purification of SUMO conjugates with **i**sobaric **t**ag for **r**elative and **a**bsolute **q**uantification (iTRAQ) mass spectrometry and an advanced method to adjust for sample-to-sample variation to study quantitatively the SUMOylation dynamics of intact *Arabidopsis* seedlings subjected to stress. Inspection of 172 SUMO substrates during and after heat shock (37ºC) revealed that stress mostly increases the abundance of existing conjugates as opposed to modifying new targets. Some of the most robustly up-regulated targets participate in RNA processing and turnover, and RNA-directed DNA modification, thus implicating SUMO as a regulator of the transcriptome during stress. Many of these targets were also strongly SUMOylated during ethanol and oxidative stress, suggesting that their modification is crucial for general stress tolerance. Collectively, our quantitative data emphasize the importance of SUMO to RNA-related processes in protecting plants from adverse environments.

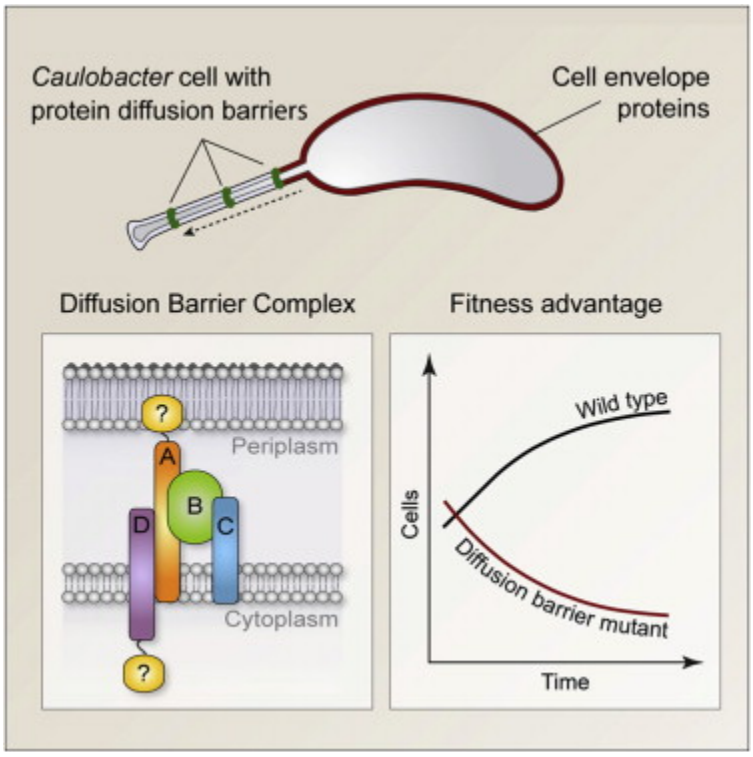
Cell

General Protein Diffusion Barriers Create Compartments within Bacterial Cells

Summary

In eukaryotes, the differentiation of cellular extensions such as cilia or neuronal axons depends on the partitioning of proteins to distinct plasma membrane domains by specialized diffusion barriers. However, examples of this compartmentalization strategy are still missing for prokaryotes, although complex cellular architectures are also widespread among this group of organisms. This study reveals the existence of a protein-mediated membrane diffusion barrier in the stalked bacterium *Caulobacter crescentus*.

We show that the *Caulobacter* cell envelope is compartmentalized by macromolecular complexes that prevent the exchange of both membrane and soluble proteins between the polar stalk extension and the cell body. The barrier structures span the cross-sectional area of the stalk and comprise at least four proteins that assemble in a cell-cycle-dependent manner. Their presence is critical for cellular fitness because they minimize the effective cell volume, allowing faster adaptation to environmental changes that require de novo synthesis of envelope proteins.



Molecular Cell

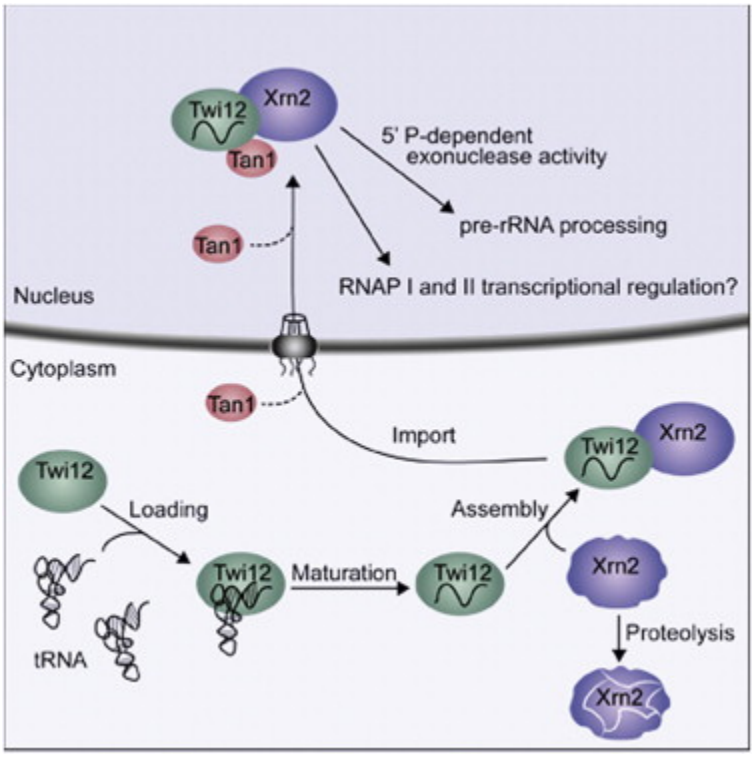
A *Tetrahymena* Piwi Bound to Mature tRNA 3′ Fragments Activates the Exonuclease Xrn2 for RNA Processing in the Nucleus

Highlights

- Twi12, an essential Piwi protein, forms a stable nuclear complex with the exonuclease Xrn2
- Twi12 is required for Xrn2 cellular accumulation, localization, and activity
- Twi12 nuclear localization requires tRNA-derived small RNA binding
- The Twi12-Xrn2 complex mediates ribosomal RNA processing

Summary

Emerging evidence suggests that Argonaute (Ago)/Piwi proteins have diverse functions in the nucleus and cytoplasm, but the molecular mechanisms employed in the nucleus remain poorly defined. The *Tetrahymena thermophila* Ago/Piwi protein Twi12 is essential for growth and functions in the nucleus. Twi12-bound small RNAs (sRNAs) are 3′ tRNA fragments that contain modified bases and thus are attenuated for base pairing to targets. We show that Twi12 assembles an unexpected complex with the nuclear exonuclease Xrn2. Twi12 functions to stabilize and localize Xrn2, as well as to stimulate its exonuclease activity. Twi12 function depends on sRNA binding, which is required for its nuclear import. Depletion of Twi12 or Xrn2 induces a cellular ribosomal RNA processing defect known to result from limiting Xrn2 activity in other organisms. Our findings suggest a role for an Ago/Piwi protein and 3′ tRNA fragments in nuclear RNA metabolism.



PLOS one

Identification of elements that dictate the specificity of mitochondrial hsp60 for its co-chaperonin.

Abstract

Type I chaperonins (cpn60/Hsp60) are essential proteins that mediate the folding of proteins in bacteria, chloroplast and mitochondria. Despite the high sequence homology among chaperonins, the mitochondrial chaperonin system has developed unique properties that distinguish it from the widely-studied bacterial system (GroEL and GroES). The most relevant difference to this study is that mitochondrial chaperonins are able to refold denatured proteins only with the assistance of the mitochondrial co-chaperonin. This is in contrast to the bacterial chaperonin, which is able to function with the help of co-chaperonin from any source. The goal of our work was to determine structural elements that govern the specificity between chaperonin and co-chaperonin pairs using mitochondrial Hsp60 as model system. We used a mutagenesis approach to obtain human mitochondrial Hsp60 mutants that are able to function with the bacterial co-chaperonin, GroES. We isolated two mutants, a single mutant (E321K) and a double mutant (R264K/E358K) that, together with GroES, were able to rescue an E. coli strain, in which the endogenous chaperonin system was silenced. Although the mutations are located in the apical domain of the chaperonin, where the interaction with co-chaperonin takes place, none of the residues are located in positions that are directly responsible for co-chaperonin binding. Moreover, while both mutants were able to function with GroES, they showed distinct functional and structural properties. Our results indicate that the phenotype of the E321K mutant is caused mainly by a profound increase in the binding affinity to all co-chaperonins, while the phenotype of R264K/E358K is caused by a slight increase in affinity toward co-chaperonins that is accompanied by an alteration in the allosteric signal transmitted upon nucleotide binding. The latter changes lead to a great increase in affinity for GroES, with only a minor increase in affinity toward the mammalian mitochondrial co-chaperonin.

A tightly regulated molecular toggle controls AAA+ disaggregase

Yuki Oguchi, Eva Kummer, Fabian Seyffer, Mykhaylo Berynskyy, Benjamin Anstett, Regina Zahn, Rebecca C Wade, Axel Mogk & Bernd Bukau

The ring-forming AAA+ protein ClpB cooperates with the DnaK chaperone system to refold aggregated proteins in *Escherichia coli*. The M domain, a ClpB-specific coiled-coil structure with two wings, motif 1 and motif 2, is essential to disaggregation, but the positioning and mechanistic role of M domains in ClpB hexamers remain unresolved. We show that M domains nestle at the ClpB ring surface, with both M-domain motifs contacting the first ATPase domain (AAA-1). Both wings contribute to maintaining a repressed ClpB activity state. Motif 2 docks intramolecularly to AAA-1 to regulate ClpB unfolding power, and motif 1 contacts a neighboring AAA-1 domain. Mutations that stabilize motif 2 docking repress ClpB, whereas

destabilization leads to derepressed ClpB activity with greater unfolding power that is toxic *in vivo*. Our results underline the vital nature of tight ClpB activity control and elucidate a regulated M-domain toggle control mechanis

Hsp70 proteins bind Hsp100 regulatory M domains to activate AAA+ disaggregase at aggregate surfaces

Fabian Seyffer, Eva Kummer, Yuki Oguchi, Juliane Winkler, Mohit Kumar, Regina Zahn, Victor Sourjik, Bernd Bukau & Axel Mogk

Abstract

Abstract **Author information Supplementary information**

Bacteria, fungi and plants rescue aggregated proteins using a powerful bichaperone system composed of an Hsp70 chaperone and an Hsp100 AAA+ disaggregase. In *Escherichia coli*, the Hsp70 chaperone DnaK binds aggregates and targets the disaggregase ClpB to the substrate. ClpB hexamers use ATP to thread substrate polypeptides through the central pore, driving disaggregation. How ClpB finds DnaK and regulates threading remains unclear. To dissect the disaggregation mechanism, we separated these steps using primarily chimeric ClpB-ClpV constructs that directly recognize alternative substrates, thereby obviating DnaK involvement. We show that ClpB has low intrinsic disaggregation activity that is normally repressed by the ClpB middle (M) domain. In the presence of aggregate, DnaK directly binds M-domain motif 2, increasing ClpB ATPase activity to unleash high ClpB threading power. Our results uncover a new function for Hsp70: the coupling of substrate targeting to AAA+ chaperone activation at aggregate surfaces.

Alternative bacterial two-component small heat shock protein systems

Abstract

Small heat shock proteins (sHsps) are molecular chaperones that prevent the aggregation of nonnative proteins. The sHsps investigated to date mostly form large, oligomeric complexes. The typical bacterial scenario seemed to be a two-component sHsps system of two homologous sHsps, such as the Escherichia coli sHsps IbpA and IbpB. With a view to expand our knowledge on bacterial sHsps, we analyzed the sHsp system of the bacterium

Deinococcus radiodurans, which is resistant against various stress conditions. D. radiodurans encodes two sHsps, termed Hsp17.7 and Hsp20.2. Surprisingly, Hsp17.7 forms only chaperone active dimers, although its crystal structure reveals the typical αcrystallin fold. In contrast, Hsp20.2 is predominantly a 36mer that dissociates into smaller oligomeric assemblies that bind substrate proteins stably. Whereas Hsp20.2 cooperates with the ATP-dependent bacterial chaperones in their refolding, Hsp17.7 keeps substrates in a refolding-competent state by transient interactions. In summary, we show that these two sHsps are strikingly different in their quaternary structures and chaperone properties, defining a second type of bacterial two-component sHsp system.