Lit Lunch - February 13, 2013

Conformational Selection in Substrate Recognition by Hsp70 Chaperones

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Abstract

Hsp70s are molecular chaperones involved in the folding and assembly of proteins. They recognize hydrophobic amino acid stretches in their substrate binding groove. However, a detailed understanding of substrate specificity is still missing. Here, we use the endoplasmic reticulum-resident Hsp70 BiP to identify binding sites in a natural client protein. Two sites are mutually recognized and form stable Hsp70–substrate complexes. In silico and in vitro analyses revealed an extended substrate conformation as a crucial factor for interaction and show an unexpected plasticity of the is conserved among different Hsp70s.

Hsp70/Hsp90 chaperone machinery is involved in the assembly of the purinosome

Jarrod B. French[a,1], Hong Zhao[a,1], Songon An[a,2], Sherry Niessen[b], Yijun Deng[a], Benjamin F. Cravatt[b,3], and Stephen J. Benkovic[a,3]

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Contributed by Stephen J. Benkovic, January 4, 2013 (sent for review December 7, 2012)

The de novo biosynthesis of purines is carried out by a highly conserved metabolic pathway that includes several validated targets for anticancer, immunosuppressant, and anti-inflammatory chemo- therapeutics.

The six enzymes in humans that catalyze the 10 chemical steps from phosphoribosylpyrophosphate to inosine monophosphate were recently shown to associate into a dynamic multiprotein com- plex called the purinosome. Here, we demonstrate that heat shock protein 90 (Hsp90), heat shock protein 70 (Hsp70), and several co- chaperones functionally colocalize with this protein complex. Knock- down of expression levels of the identified cochaperones leads to disruption of purinosomes. In addition, small molecule inhibitors of Hsp90 and Hsp70 reversibly disrupt purinosomes and are shown to have a synergistic effect with methotrexate, an anticancer agent that targets purine biosynthesis. These data implicate the Hsp90/Hsp70 chaperone machinery in the assembly of the purinosome and provide a strategy for the development of improved anticancer therapies that disrupt purine biosynthesis.

Nature and Structural and Molecular Biology

Interaction between FIP200 and ATG16L1 distinguishes ULK1 complex–dependent and –independent autophagy

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Autophagy is a finely orchestrated cellular catabolic process that requires multiple autophagy-related gene products (ATG proteins). The ULK1 complex functions to integrate upstream signals to downstream ATG proteins through an unknown mechanism. Here we have identified an interaction between mammalian FIP200 and ATG16L1, essential components of the ULK1 and ATG5 complexes, respectively. Further analyses show this is a direct interaction mediated by a short domain of ATG16L1 that we term the FIP200-binding domain (FBD). The FBD is not required for ATG16L1 self-dimerization or interaction with ATG5. Notably, an FBD-deleted ATG16L1 mutant is defective in mediating amino acid starvation–induced autophagy, which requires the ULK1 complex. However, this mutant retains its function in supporting glucose deprivation–induced autophagy, a ULK1 complex–independent process. This study therefore identifies a previously uncharacterized interaction between the ULK1 and ATG5 complexes that can distinguish ULK1-dependent and -independent autophagy processes.

Complexes of HIV-1 RT, NNRTI and RNA/DNA hybrid reveal a structure compatible with RNA degradation

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Hundreds of structures of type 1 human immunodeficiency virus (HIV-1) reverse transcriptase (RT) have been determined, but only one contains an RNA/DNA hybrid. Here we report three structures of HIV-1 RT complexed with a non-nucleotide RT inhibitor (NNRTI) and an RNA/DNA hybrid. In the presence of an NNRTI, the RNA/DNA structure differs from all prior nucleic acid–RT structures including the RNA/DNA hybrid. The enzyme structure also differs from all previous RT–DNA complexes. Thus, the hybrid has ready access to the RNase-H active site. These observations indicate that an RT–nucleic acid complex may adopt two structural states, one competent for DNA polymerization and the other for RNA degradation. RT mutations that confer drug resistance but are distant from the inhibitor-binding sites often map to the unique RT-hybrid interface that undergoes conformational changes between two catalytic states.

Journal of Biological Chemistry

Glycosyltransferases from Oat (*Avena*) Implicated in the Acylation of Avenacins

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Plants produce a huge array of specialized metabolites that have important functions in defense against biotic and abiotic stresses. Many of these compounds are glycosylated by family 1 glycosyltransferases (GTs). Oats (Avena spp.) make root-derived antimicrobial triterpenes (avenacins) that provide protection against soil-borne diseases. The ability to synthesize avenacins has evolved since the divergence of oats from other cereals and grasses. The major avenacin, A-1, is acylated with N-methylanthranilic acid. Previously, we have cloned and characterized three genes for avenacin synthesis (for the triterpene synthase SAD1, a triterpene-modifying cytochrome P450 SAD2, and the serine carboxypeptidase-like acyl transferase SAD7), which form part of a biosynthetic gene cluster. Here, we identify a fourth member of this gene cluster encoding a GT belonging to clade L of family 1 (UGT74H5), and show that this enzyme is an N-methylanthranilic acid O-glucosyltransferase implicated in the synthesis of avenacin A-1. Two other closely related family 1 GTs (UGT74H6 and UGT74H7) are also expressed in oat roots. One of these (UGT74H6) is able to glucosylate both N-methylanthranilic acid and benzoic acid, whereas the function of the other (UGT74H7) remains unknown. Our investigations indicate that UGT74H5 is likely to be key for the generation of the activated acyl donor used by SAD7 in the synthesis of the major avenacin, A-1, whereas UGT74H6 may contribute to the synthesis of other forms of avenacin that are acylated with benzoic acid.

NatureBiotechnology

Epigenetic trigger for tomato ripening

JosephREcker

Plant cell miR156 and miR390 Regulate tasiRNA Accumulation and Developmental Timing in Physcomitrella patens

Sung Hyun Choa, Ceyda Coruha,b and Michael J. Axtell

Abstract

microRNA156 (miR156) affects developmental timing in flowering plants. miR156 and its target relationships with members of the SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) gene family appear universally conserved in land plants, but the specific functions of miR156 outside of flowering plants are unknown. We find that miR156 promotes a developmental change from young filamentous protonemata to leafy gametophores in the moss Physcomitrella patens, opposite to its role as an inhibitor of development in flowering plants. P. patens miR156 also influences accumulation of trans-acting small interfering RNAs (tasiRNAs) dependent upon a second ancient microRNA, miR390. Both miR156 and miR390 directly target a single major tasiRNA primary transcript. Inhibition of miR156 function causes increased miR390-triggered tasiRNA accumulation and decreased accumulation of tasiRNA targets. Overexpression of miR390 also caused a slower formation of gametophores, elevated

miR390-triggered tasiRNA accumulation, and reduced level of tasiRNA targets. We conclude that a gene regulatory network controlled by miR156, miR390, and their targets controls developmental change in P. patens. The broad outlines and regulatory logic of this network are conserved in flowering plants, albeit with some modifications. Partially conserved small RNA networks thus influence developmental timing in plants with radically different body plans.

Sha Yu, Vinicius C. Galvão, Yan-Chun Zhang, Daniel Horrer, Tian-Qi Zhang, Yan-Hong Hao, Yu-Qi Feng, Shui Wang, Markus Schmid, and Jia-Wei Wang

Gibberellin Regulates the Arabidopsis Floral Transition through miR156-Targeted SQUAMOSA PROMOTER BINDING?LIKE Transcription Factors

Abstract

Gibberellin (GA), a diterpene hormone, plays diverse roles in plant growth and development, including seed germination, stem elongation, and flowering time. Although it is known that GA accelerates flowering through degradation of transcription repressors, DELLAs, the underlying mechanism is poorly understood. We show here that DELLA directly binds to microRNA156 (miR156)-targeted SQUAMOSA PROMOTER BINDING?LIKE (SPL) transcription factors, which promote flowering by activating miR172 and MADS box genes. The interaction between DELLA and SPL interferes with SPL transcriptional activity and consequently delays floral transition through inactivating miR172 in leaves and MADS box genes at shoot apex under long-day conditions or through repressing MADS box genes at the shoot apex under short-day conditions. Our results elucidate the molecular mechanism by which GA controls flowering and provide the missing link between DELLA and MADS box genes.
A Dominant Point Mutation in a RINGv E3 Ubiquitin Ligase Homoeologous Gene Leads to Cleistogamy in Brassica napus\

Yun-Hai Lu, Dominique Arnaud, Harry Belcram, Cyril Falentin, Patricia Rouault, Nathalie Piel, Marie-Odile Lucas, Jérémy Just, Michel Renard, Régine Delourme, and Boulos Chalhoub

Abstract

In the allopolyploid Brassica napus, we obtained a petal-closed flower mutation by ethyl methanesulfonate mutagenesis. Here, we report cloning and characterization of the Bn-CLG1A (CLG for cleistogamy) gene and the Bn-clg1A-1D mutant allele responsible for the cleistogamy phenotype. Bn-CLG1A encodes a RINGv E3 ubiquitin ligase that is highly conserved across eukaryotes. In the Bn-clg1A-1D mutant allele, a C-to-T transition converts a Pro at position 325 to a Leu (P325L), causing a dominant mutation leading to cleistogamy. B. napus and Arabidopsis thaliana plants transformed with a Bn-clg1A-1D allele show cleistogamous flowers, and characterization of these flowers suggests that the Bn-clg1A-1D mutation causes a pronounced negative regulation of cutin biosynthesis or loading and affects elongation or differentiation of petal and sepal cells. This results in an inhibition or a delay of petal development, leading to folded petals. A homoeologous gene (Bn-CLG1C), which shows 99.5% amino

acid identity and is also constitutively and equally expressed to the wild-type Bn-CLG1A gene, was also identified. We showed that P325L is not a loss-of-function mutation and did not affect expression of Bn-clg1A-1D or Bn-CLG1C. Our findings suggest that P325L is a gain-of-function semidominant mutation, which led to either hyper- or neofunctionalization of a redundant homoeologous gene.

The SWI2/SNF2 Chromatin Remodeling ATPase BRAHMA Represses Abscisic Acid Responses in the Absence of the Stress Stimulus in Arabidopsis

Soon-Ki Han, Yi Sang, Americo Rodrigues, BIOL425 F2010, Miin-Feng Wu, Pedro L. Rodriguez, and Doris Wagner

The survival of plants as sessile organisms depends on their ability to cope with environmental challenges. Of key importance in this regard is the phytohormone abscisic acid (ABA). ABA not only promotes seed dormancy but also triggers growth arrest in postgermination embryos that encounter water stress. This is accompanied by increased desiccation tolerance. Postgermination ABA responses in Arabidopsis thaliana are mediated in large part by the ABA-induced basic domain/leucine zipper transcription factor ABA INSENSITIVE5 (ABI5). Here, we show that loss of function of the SWI2/SNF2 chromatin remodeling ATPase BRAHMA (BRM) causes ABA hypersensitivity during postgermination growth arrest. ABI5 expression was derepressed in brm mutants in the absence of exogenous ABA and accumulated to high levels upon ABA sensing. This effect was likely direct; chromatin immunoprecipitation revealed BRM binding to the ABI5 locus. Moreover, loss of BRM activity led to destabilization of a nucleosome likely to repress ABI5 transcription. Finally, the abi5 null mutant was epistatic to BRM in postgermination growth arrest. In addition, vegetative growth defects typical of brm mutants in the absence of ABA treatment could be partially overcome by reduction of ABA responses, and brm mutants displayed increased drought tolerance. We propose a role for BRM in the balance between growth or stress responses.

A MAPK Cascade Downstream of ERECTA Receptor-Like Protein Kinase Regulates Arabidopsis Inflorescence Architecture by Promoting Localized Cell Proliferation

Xiangzong Meng, Huachun Wang, Yunxia He, Yidong Liu, John C. Walker, Keiko U. Torii, and Shuqun Zhang

Spatiotemporal-specific cell proliferation and cell differentiation are critical to the formation of normal tissues, organs, and organisms. The highly coordinated cell differentiation and proliferation events illustrate the importance of cell?cell communication during growth and development. In Arabidopsis thaliana, ERECTA (ER), a receptor-like protein kinase, plays important roles in promoting localized cell proliferation, which determines inflorescence architecture, organ shape, and size. However, the downstream signaling components remain unidentified. Here, we report a mitogen-activated protein kinase (MAPK; or MPK) cascade that functions downstream of ER in regulating localized cell proliferation. Similar to an er mutant, loss of function of MPK3/MPK6 or their upstream MAPK kinases (MAPKKs; or MKKs), MKK4/MKK5, resulted in shortened pedicels and clustered inflorescences. Epistasis

analysis demonstrated that the gain of function of MKK4 and MKK5 transgenes could rescue the loss-of-function er mutant phenotype at both morphological and cellular levels, suggesting that the MPK3/MPK6 cascade functions downstream of the ER receptor. Furthermore, YODA (YDA), a MAPKK kinase, was shown to be upstream of MKK4/MKK5 and downstream of ER in regulating inflorescence architecture based on both gain- and loss-of-function data. Taken together, these results suggest that the YDA-MKK4/MKK5-MPK3/MPK6 cascade functions downstream of the ER receptor in regulating localized cell proliferation, which further shapes the morphology of plant organs.

UBIQUITIN-SPECIFIC PROTEASE16 Modulates Salt Tolerance in Arabidopsis by Regulating Na+/H+ Antiport Activity and Serine Hydroxymethyltransferase Stability

Huapeng Zhou, Jinfeng Zhao, Yongqing Yang, Changxi Chen, Yanfen Liu, Xuehua Jin, Limei Chen, Xueyong Li, Xing Wang Deng, Karen S. Schumaker, and Yan Guo

Protein ubiquitination is a reversible process catalyzed by ubiquitin ligases and ubiquitin-specific proteases (UBPs). We report the identification and characterization of UBP16 in Arabidopsis thaliana. UBP16 is a functional ubiquitin-specific protease and its enzyme activity is required for salt tolerance. Plants lacking UBP16 were hypersensitive to salt stress and accumulated more sodium and less potassium. UBP16 positively regulated plasma membrane Na+/H+ antiport activity. Through yeast two-hybrid screening, we identified a putative target of UBP16, SERINE HYDROXYMETHYLTRANSFERASE1 (SHM1), which has previously been reported to be involved in photorespiration and salt tolerance in Arabidopsis. We found that SHM1 is degraded in a 26S proteasome?dependent process, and UBP16 stabilizes SHM1 by removing the conjugated ubiquitin. Ser hydroxymethyltransferase activity is lower in the ubp16 mutant than in the wild type but higher than in the shm1 mutant. During salt stress, UBP16 and SHM1 function in preventing cell death and reducing reactive oxygen species accumulation, activities that are correlated with increasing Na+/H+ antiport activity. Overexpression of SHM1 in the ubp16 mutant partially rescues its salt-sensitive phenotype. Taken together, our results suggest that UBP16 is involved in salt tolerance in Arabidopsis by modulating sodium transport activity and repressing cell death at least partially through modulating SMH1stability and activity.

Pipecolic Acid, an Endogenous Mediator of Defense Amplification and Priming, Is a Critical Regulator of Inducible Plant Immunity

Hana Návarová, Friederike Bernsdorff, Anne-Christin Döring, and Jürgen Zeier

Metabolic signals orchestrate plant defenses against microbial pathogen invasion. Here, we report the identification of the non-protein amino acid pipecolic acid (Pip), a common Lys catabolite in plants and animals, as a critical regulator of inducible plant immunity. Following pathogen recognition, Pip accumulates in inoculated Arabidopsis thaliana leaves, in leaves distal from the site of inoculation, and, most specifically, in petiole exudates from inoculated leaves. Defects of mutants in AGD2-LIKE DEFENSE RESPONSE PROTEIN1 (ALD1) in systemic acquired resistance (SAR) and in basal, specific, and ?-aminobutyric acid?induced resistance to bacterial infection are associated with a lack of Pip production.

Exogenous Pip complements these resistance defects and increases pathogen resistance of wild-type plants. We conclude that Pip accumulation is critical for SAR and local resistance to bacterial pathogens. Our data indicate that biologically induced SAR conditions plants to more effectively synthesize the phytoalexin camalexin, Pip, and salicylic acid and primes plants for early defense gene expression. Biological priming is absent in the pipecolate-deficient ald1 mutants. Exogenous pipecolate induces SAR-related defense priming and partly restores priming responses in ald1. We conclude that Pip orchestrates defense amplification, positive regulation of salicylic acid biosynthesis, and priming to guarantee effective local resistance induction and the establishment of SAR.

Calcium Channels and Acquired Thermotolerance: Here Comes the Sun and It?s All Right JenniferMach

Plasma Membrane Cyclic Nucleotide Gated Calcium Channels Control Land Plant Thermal Sensing and AcquiredThermotolerance

Andrija Finka, America Farinia Henriquez Cuendet, Frans J.M. Maathuis, Younousse Saidi, and Pierre Goloubinoff

Abstract

Typically at dawn on a hot summer day, land plants need precise molecular thermometers to sense harmless increments in the ambient temperature to induce a timely heat shock response (HSR) and accumulate protective heat shock proteins in anticipation of harmful temperatures at mid-day. Here, we found that the cyclic nucleotide gated calcium channel (CNGC) CNGCb gene from Physcomitrella patens and its Arabidopsis thaliana ortholog CNGC2, encode a component of cyclic nucleotide gated Ca2+ channels that act as the primary thermosensors of land plant cells. Disruption of CNGCb or CNGC2 produced a hyper-thermosensitive phenotype, giving rise to an HSR and acquired thermotolerance at significantly milder heat-priming treatments than in wild-type plants. In an aequorin-expressing moss, CNGCb loss-of-function caused a hyper-thermoresponsive Ca2+ influx and altered Ca2+ signaling. Patch clamp recordings on moss protoplasts showed the presence of three distinct thermoresponsive Ca2+ channels in wild-type cells. Deletion of CNGCb led to a total absence of one and increased the open probability of the remaining two thermoresponsive Ca2+ channels. Thus, CNGC2 and CNGCb are expected to form heteromeric Ca2+ channels with other related CNGCs. These channels in the plasma membrane respond to increments in the ambient temperature by triggering an optimal HSR, leading to the onset of plant acquired thermotolerance.

Minsoo Kim, Ung Lee, Ian Small, Catherine Colas des Francs-Small, and Elizabeth Vierling

Mutations in an Arabidopsis Mitochondrial Transcription Termination Factor?Related Protein Enhance Thermotolerance in the Absence of the Major Molecular Chaperone HSP101

The molecular chaperone heat shock protein101 (HSP101) is required for acquired thermotolerance in

plants and other organisms. To identify factors that interact with HSP101 or that are involved in thermotolerance, we screened for extragenic suppressors of a dominant-negative allele of Arabidopsis thaliana HSP101, hot1-4. One suppressor, shot1 (for suppressor of hot1-4 1), encodes a mitochondrial transcription termination factor (mTERF)?related protein, one of 35 Arabidopsis mTERFs about which there is limited functional data. Missense (shot1-1) and T-DNA insertion (shot1-2) mutants suppress the hot1-4 heat-hypersensitive phenotype. Furthermore, shot1-2 suppresses other heat-sensitive mutants, and shot1-2 alone is more heat tolerant than the wild type. SHOT1 resides in mitochondria, indicating it functions independently of cytosolic/nuclear HSP101. Microarray analysis suggests altered mitochondrial function and/or retrograde signaling in shot1-2 increases transcripts of other HSPs and alters expression of redox-related genes. Reduced oxidative damage is the likely cause of shot1 thermotolerance, indicating HSP101 repairs protein oxidative damage and/or reduced oxidative damage allows recovery in the absence of HSP101. Changes in organelle-encoded transcripts in shot1 demonstrate that SHOT1 is involved in organelle gene regulation. The heat tolerance of shot1 emphasizes the importance of mitochondria in stress tolerance, and defining its function may provide insights into control of oxidative damage for engineering stress-resistant plants.

Emerging Roots Alter Epidermal Cell Fate through Mechanical and Reactive Oxygen Species Signaling

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Abstract

A central question in biology is how spatial information is conveyed to locally establish a developmental program. Rice (Oryza sativa) can survive flash floods by the emergence of adventitious roots from the stem. Epidermal cells that overlie adventitious root primordia undergo cell death to facilitate root emergence. Root growth and epidermal cell death are both controlled by ethylene. This study aimed to identify the signal responsible for the spatial control of cell death. Epidermal cell death correlated with the proximity to root primordia in wild-type and ADVENTITIOUS ROOTLESS1 plants, indicating that the root emits a spatial signal. Ethylene-induced root growth generated a mechanical force of ?18 millinewtons within 1 h. Force application to epidermal cells above root primordia caused cell death in a dose-dependent manner and was inhibited by 1-methylcyclopropene or diphenylene iodonium, an inhibitor of NADPH oxidase. Exposure of epidermal cells not overlying a root to either force and ethylene or force and the catalase inhibitor aminotriazole induced ectopic cell death. Genetic downregulation of the reactive oxygen species (ROS) scavenger METALLOTHIONEIN2b likewise promoted force-induced ectopic cell death. Hence, reprogramming of epidermal cell fate by the volatile plant hormone ethylene requires two signals: mechanosensing for spatial resolution and ROS for cell death signaling.

PLant Journal

Apical myosin XI anticipates F-actin during polarized growth of Physcomitrella patens cells

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Summary
Tip growth is essential for land colonization by bryophytes, plant sexual reproduction and water and nutrient uptake. Because this specialized form of polarized cell growth requires both a dynamic actin cytoskeleton and active secretion, it has been proposed that the F-actin-associated motor myosin XI is essential for this process. Nevertheless, a spatial and temporal relationship between myosin XI and F-actin during tip growth is not known in any plant cell. Here, we use the highly polarized cells of the moss Physcomitrella patens to show that myosin XI and F-actin localize, in vivo, at the same apical domain and that both signals fluctuate. Surprisingly, phase analysis shows that increase in myosin XI anticipates that of F-actin; in contrast, myosin XI levels at the tip fluctuate in identical phase with a vesicle marker. Pharmacological analysis using a low concentration of the actin polymerization inhibitor latrunculin B showed that the F-actin at the tip can be significantly diminished while myosin XI remains elevated in this region, suggesting that a mechanism exists to cluster myosin XI-associated structures at the cell's apex. In addition, this approach uncovered a mechanism for actin polymerization-dependent motility in the moss cytoplasm, where myosin XI-associated structures seem to anticipate and organize the actin polymerization machinery. From our results, we inferred a model where the interaction between myosin XI-associated vesicular structures and F-actin polymerization-driven motility function at the cell's apex to maintain polarized cell growth. We hypothesize this is a general mechanism for the participation of myosin XI and F-actin in tip growing cells.

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Aggravating genetic interactions allow a solution to redundancy in a bacterial pathogen.

O'Connor TJ, Boyd D, Dorer MS, Isberg RR.

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Abstract

Interactions between hosts and pathogens are complex, so understanding the events that govern these interactions requires the analysis of molecular mechanisms operating in both organisms. Many pathogens use multiple strategies to target a single event in the disease process, confounding the identification of the important determinants of virulence. We developed a genetic screening strategy called insertional mutagenesis and depletion (iMAD) that combines bacterial mutagenesis and RNA interference, to systematically dissect the interplay between a pathogen and its host. We used this technique to resolve the network of proteins secreted by the bacterium Legionella pneumophila to promote intracellular growth, a critical determinant of pathogenicity of this organism. This strategy is broadly applicable, allowing the dissection of any interface between two organisms involving numerous interactions.

PMID: 23239729 [PubMed - indexed for MEDLINE]

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Genome-wide detection of single-nucleotide and copy-number variations of a single human cell.

Zong C, Lu S, Chapman AR, Xie XS.

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Abstract

Kindred cells can have different genomes because of dynamic changes in DNA. Single-cell sequencing is needed to characterize these genomic differences but has been hindered by whole-genome amplification bias, resulting in low genome coverage. Here, we report on a new amplification method-multiple annealing and looping-based amplification cycles (MALBAC)-that offers high uniformity across the genome. Sequencing MALBAC-amplified DNA achieves 93% genome coverage ≥1x for a single human cell at 25x mean sequencing depth.

We detected digitized copy-number variations (CNVs) of a single cancer cell. By sequencing three kindred cells, we were able to identify individual single-nucleotide variations (SNVs), with no false positives detected. We directly measured the genome-wide mutation rate of a cancer cell line and found that purine-pyrimidine exchanges occurred unusually frequently among the newly acquired SNVs.

PMID: 23258894 [PubMed - indexed for MEDLINE]

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Translation elongation factor EF-P alleviates ribosome stalling at polyproline stretches.

Ude S, Lassak J, Starosta AL, Kraxenberger T, Wilson DN, Jung K.

Center for Integrated Protein Science Munich (CiPSM), Ludwig-Maximilians-Universität München, Munich, Germany.

Comment in

- Biochemistry. Getting past polyproline pauses. [Science. 2013]

Abstract

Translation elongation factor P (EF-P) is critical for virulence in bacteria. EF-P is present in all bacteria and orthologous to archaeal and eukaryotic initiation factor 5A, yet the biological function has so far remained enigmatic. Here, we demonstrate that EF-P is an elongation factor that enhances translation of polyproline-containing proteins: In the absence of EF-P, ribosomes stall at polyproline stretches, whereas the presence of EF-P alleviates the translational stalling. Moreover, we demonstrate the physiological relevance of EF-P to fine-tune the expression of the polyproline-containing pH receptor CadC to levels necessary for an appropriate stress response. Bacterial, archaeal, and eukaryotic cells have hundreds to thousands of polyproline-containing proteins of diverse function, suggesting that EF-P and a/eIF-5A are critical for copy-number adjustment of multiple pathways across all kingdoms of life.

PMID: 23239623 [PubMed - indexed for MEDLINE]

Related citations

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EF-P is essential for rapid synthesis of proteins containing consecutive proline residues.

Doerfel LK, Wohlgemuth I, Kothe C, Peske F, Urlaub H, Rodnina MV.

Department of Physical Biochemistry, Max Planck Institute for Biophysical Chemistry, Goettingen, Germany.

Comment in

- Biochemistry. Getting past polyproline pauses. [Science. 2013]

Abstract

Elongation factor P (EF-P) is a translation factor of unknown function that has been implicated in a great variety of cellular processes. Here, we show that EF-P prevents ribosome from stalling during synthesis of proteins containing consecutive prolines, such as PPG, PPP, or longer proline strings, in natural and engineered model proteins. EF-P promotes peptide-bond formation and stabilizes the peptidyl-transfer RNA in the catalytic center of the ribosome. EF-P is posttranslationally modified by a hydroxylated β-lysine attached to a lysine residue. The modification enhances the catalytic proficiency of the factor mainly by increasing its affinity to the ribosome. We propose that EF-P and its eukaryotic homolog, eIF5A, are essential for the synthesis of a subset of proteins containing proline stretches in all cells.

PMID: 23239624 [PubMed - indexed for MEDLINE]

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Science AAAS

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Para-aminosalicylic acid acts as an alternative substrate of folate metabolism in Mycobacterium tuberculosis.

Chakraborty S, Gruber T, Barry CE 3rd, Boshoff HI, Rhee KY.

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Abstract

Folate biosynthesis is an established anti-infective target, and the antifolate para-aminosalicylic acid (PAS) was one of the first anti-infectives introduced into clinical practice on the basis of target-based drug discovery. Fifty years later, PAS continues to be used to treat tuberculosis. PAS is assumed to inhibit dihydropteroate synthase (DHPS) in Mycobacterium tuberculosis by mimicking the substrate p-aminobenzoate (PABA). However, we found that sulfonamide inhibitors of DHPS inhibited growth of M. tuberculosis only weakly because of their intracellular metabolism. In contrast, PAS served as a replacement substrate for DHPS. Products of PAS metabolism at this and subsequent steps in folate metabolism inhibited those enzymes, competing with their substrates. PAS is thus a prodrug that blocks growth of M. tuberculosis when its active forms are generated by enzymes in the pathway they poison.

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Related citations

Science

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