Year of publication 2019

Judith Souphron, Satish Bodakuntla, A. S. Jijumon, Goran Lakisic, Alexis M. Gautreau, Carsten Janke, Maria M. Magiera (2019 Apr 17)

Purification of tubulin with controlled post-translational modifications by polymerization–depolymerization cycles

Journal of cell science

Summary

In vitro reconstitutions of microtubule assemblies have provided essential mechanistic insights into the molecular bases of microtubule dynamics and their interactions with associated proteins. The tubulin code has emerged as a regulatory mechanism for microtubule functions, which suggests that tubulin isotypes and post-translational modifications (PTMs) play important roles in controlling microtubule functions. To investigate the tubulin code mechanism, it is essential to analyze different tubulin variants in vitro. Until now, this has been difficult, as most reconstitution experiments have used heavily post-translationally modified tubulin purified from brain tissue. Therefore, we developed a protocol that allows purification of tubulin with controlled PTMs from limited sources through cycles of polymerization and depolymerization. Although alternative protocols using affinity purification of tubulin also yield very pure tubulin, our protocol has the unique advantage of selecting for fully functional tubulin, as non-polymerizable tubulin is excluded in the successive polymerization cycles. It thus provides a novel procedure for obtaining tubulin with controlled PTMs for in vitro reconstitution experiments. We describe specific procedures for tubulin purification from adherent cells, cells grown in suspension cultures and single mouse brains. The protocol can be combined with drug treatment, transfection of cells before tubulin purification or enzymatic treatment during the purification process. The amplification of cells and their growth in spinner bottles takes ~13 d; the tubulin purification takes 6–7 h. The tubulin can be used in total internal reflection fluorescence (TIRF)-microscopy-based experiments or pelleting assays for the investigation of intrinsic properties of microtubules and their interactions with associated proteins.

https://www.nature.com/articles/s41596-019-0153-7

Emiliano Roselli, Paula Araya, Nicolás Gonzalo Núñez, Gerardo Gatti, Francesca Graziano, Christine Sedlik, Philippe Benaroch, Eliane Piaggio, Mariana Maccioni (2019 Apr 6)

TLR3 Activation of Intratumoral CD103 Dendritic Cells Modifies the Tumor Infiltrate Conferring Anti-tumor Immunity.

Frontiers in immunology : 503 : DOI : 10.3389/fimmu.2019.00503

Summary

An important challenge in cancer immunotherapy is to expand the number of patients that benefit from immune checkpoint inhibitors (CI), a fact that has been related to the pre-existence of an efficient anti-tumor immune response. Different strategies are being

proposed to promote tumor immunity and to be used in combined therapies with CI. Recently, we reported that intratumoral administration of naked poly A:U, a dsRNA mimetic empirically used in early clinical trials with some success, delays tumor growth and prolongs mice survival in several murine cancer models. Here, we show that CD103 cDC1 and, to a much lesser extent CD11b cDC2, are the only populations expressing TLR3 at the tumor site, and consequently could be potential targets of poly A:U. Upon poly A:U administration these cells become activated and elicit profound changes in the composition of the tumor immune infiltrate, switching the immune suppressive tumor environment to anti-tumor immunity. The sole administration of naked poly A:U promotes striking changes within the lymphoid compartment, with all the anti-tumoral parameters being enhanced: a higher frequency of CD8 Granzyme B T cells, (lower Treg/CD8 ratio) and an important expansion of tumor-antigen specific CD8 T cells. Also, PD1/PDL1 showed an increased expression indicating that neutralization of this axis could be exploited in combination with poly A:U. Our results shed new light to promote further assays in this dsRNA mimetic to the clinical field.

Katerina Duskova, Jérémy Lamarche, Souheila Amor, Coralie Caron, Nicolas Queyriaux, Marie Gaschard, Marie-Jose Penouilh, Guillaume De Robillard, Dominique Delmas, Charles H Devillers, Anton Granzhan, Marie-Paule Teulade-Fichou, Murielle Chavarot-Kerlidou, Bruno Therrien, Sébastien Britton, David Monchaud (2019 Apr 3)

Identification of three-way DNA junction ligands through screening of chemical libraries and validation by complementary in vitro assays.

Journal of medicinal chemistry : Just Accepted Manuscript : DOI : 10.1021/acs.jmedchem.8b01978

Summary

The human genome is replete with repetitive DNA sequences that can fold into thermodynamically stable secondary structures such as hairpins and quadruplexes. Cellular enzymes exist to cope with these structures whose stable accumulation would result in DNA damage through interference with DNA transactions such as transcription and replication. Therefore, chemical stabilization of secondary DNA structures offers an attractive way to foster DNA transaction-associated damages to trigger cell death in proliferating cancer cells. While much emphasis has been recently given to DNA quadruplexes, we focused here on three-way DNA junctions (TWJ) and report on a strategy to identify TWJ-targeting agents through a combination of in vitro techniques (TWJ-Screen, PAGE, FRET-melting, ESI-MS, dialysis equilibrium and SRB assays). We designed a complete workflow and screened 1200 compounds to identify promising TWJ-ligands selected on stringent criteria in terms of TWJ folding ability, affinity and selectivity.

Marianne Burbage, Marine Gros, Sebastian Amigorena (2019 Apr 3)

Translate less, prime better, to improve anti-tumor responses.

Nature immunology : DOI : 10.1038/s41590-019-0371-8

Summary

David Partouche, Jérémie Mathurin, Antoine Malabirade, Sergio Marco, Christophe Sandt, Véronique Arluison, Ariane Deniset-Besseau, Sylvain Trépout (2019 Apr 1)

Correlative infrared nanospectroscopy and transmission electron microscopy to investigate nanometric amyloid fibrils: prospects and challenges.

Journal of microscopy : 274 : 23-31 : DOI : 10.1111/jmi.12779

Summary

Propagation of structural information through conformational changes in host-encoded amyloid proteins is at the root of many neurodegenerative disorders. Although important breakthroughs have been made in the field, fundamental issues like the 3D-structures of the fibrils involved in some of those disorders are still to be elucidated. To better characterise those nanometric fibrils, a broad range of techniques is currently available. Nevertheless none of them is able to perform direct chemical characterisation of single protein fibrils. In this work, we propose to investigate the structure of the C-terminal region of a bacterial protein called Hfq as a model amyloidogenic protein, using a correlative approach. The complementary techniques used are transmission electron microscopy and a newly developed infrared nanospectroscopy technique called AFM-IR. We introduce and discuss the strategy that we have implemented as well as the protocol, challenges and difficulties encountered during this study to characterise amyloid assemblies at the nearly single-molecule level. LAY DESCRIPTION: Propagation of structural information through conformational changes in amyloid proteins is at the root of many neurodegenerative disorders. Amyloids are nanostructures originating from the aggregation of multiple copies of peptide or protein monomers that eventually form fibrils. Often described as being the cause for the development of various diseases, amyloid fibrils are of major significance in the public health domain. While important breakthroughs have been made in the field, fundamental issues like the 3D-structures of the fibrils implied in some of those disorders are still to be elucidated. To better characterise these fibrils, a broad range of techniques is currently available for the detection and visualisation of amyloid nanostructures. Nevertheless none of them is able to perform direct chemical characterisation of single protein fibrils. In this work, we propose to investigate the structure of model amyloidogenic fibrils using a correlative approach. The complementary techniques used are transmission electron microscopy and a newly developed infrared nanospectroscopy technique called AFM-IR that allows chemical characterisation at the nanometric scale. The strategy, protocol, challenges and difficulties encountered in this approach are introduced and discussed herein.

Matthieu Gratia, Mathieu P Rodero, Cécile Conrad, Elias Bou Samra, Mathieu Maurin, Gillian I Rice, Darragh Duffy, Patrick Revy, Florence Petit, Russell C Dale, Yanick J Crow, Mounira Amor-Gueret, Nicolas Manel (2019 Apr 1)

Bloom syndrome protein restrains innate immune sensing of micronuclei by cGAS.

The Journal of experimental medicine : DOI : jem.20181329

Summary

Cellular innate immune sensors of DNA are essential for host defense against invading pathogens. However, the presence of self-DNA inside cells poses a risk of triggering unchecked immune responses. The mechanisms limiting induction of inflammation by self-DNA are poorly understood. BLM RecQ-like helicase is essential for genome integrity and is deficient in Bloom syndrome (BS), a rare genetic disease characterized by genome instability, accumulation of micronuclei, susceptibility to cancer, and immunodeficiency. Here, we show that BLM-deficient fibroblasts show constitutive up-regulation of inflammatory interferon-stimulated gene (ISG) expression, which is mediated by the cGAS-STING-IRF3 cytosolic DNA-sensing pathway. Increased DNA damage or down-regulation of the cytoplasmic exonuclease TREX1 enhances ISG expression in BLM-deficient fibroblasts. cGAS-containing cytoplasmic micronuclei are increased in BS cells. Finally, BS patients demonstrate elevated ISG expression in peripheral blood. These results reveal that BLM limits ISG induction, thus connecting DNA damage to cellular innate immune response, which may contribute to human pathogenesis.

Dimitra Kerdidani, Panagiotis Chouvardas, Ares Rocanin Arjo, Ioanna Giopanou, Giannoula Ntaliarda, Yu Amanda Guo, Mary Tsikitis, Georgios Kazamias, Konstantinos Potaris, Georgios T Stathopoulos, Spyros Zakynthinos, Ioannis Kalomenidis, Vassili Soumelis, George Kollias, Maria Tsoumakidou (2019 Mar 31)

Wnt1 silences chemokine genes in dendritic cells and induces adaptive immune resistance in lung adenocarcinoma.

Nature communications : 1405 : DOI : 10.1038/s41467-019-09370-z

Summary

Lung adenocarcinoma (LUAD)-derived Wnts increase cancer cell proliferative/stemness potential, but whether they impact the immune microenvironment is unknown. Here we show that LUAD cells use paracrine Wnt1 signaling to induce immune resistance. In TCGA, Wnt1 correlates strongly with tolerogenic genes. In another LUAD cohort, Wnt1 inversely associates with T cell abundance. Altering Wnt1 expression profoundly affects growth of murine lung adenocarcinomas and this is dependent on conventional dendritic cells (cDCs) and T cells. Mechanistically, Wnt1 leads to transcriptional silencing of CC/CXC chemokines in cDCs, T cell exclusion and cross-tolerance. Wnt-target genes are up-regulated in human intratumoral cDCs and decrease upon silencing Wnt1, accompanied by enhanced T cell cytotoxicity. siWnt1-nanoparticles given as single therapy or part of combinatorial immunotherapies act at both arms of the cancer-immune ecosystem to halt tumor growth. Collectively, our studies show that Wnt1 induces immunologically cold tumors through cDCs and highlight its immunotherapeutic targeting.

Matteo Gentili, Xavier Lahaye, Francesca Nadalin, Guilherme P F Nader, Emilia Puig Lombardi, Solène Herve, Nilushi S De Silva, Derek C Rookhuizen, Elina Zueva, Christel Goudot, Mathieu

Maurin, Aurore Bochnakian, Sebastian Amigorena, Matthieu Piel, Daniele Fachinetti, Arturo Londoño-Vallejo, Nicolas Manel (2019 Mar 28)
The N-Terminal Domain of cGAS Determines Preferential Association with Centromeric DNA and Innate Immune Activation in the Nucleus.
Cell reports : 3798 : DOI : S2211-1247(19)30365-1

Summary

Daisuke Inoue, Dorian Obino, Judith Pineau, Francesca Farina, Jérémie Gaillard, Christophe Guerin, Laurent Blanchoin, Ana-Maria Lennon-Duménil, Manuel Théry (2019 Mar 24)
Actin filaments regulate microtubule growth at the centrosome.
The EMBO journal : DOI : e99630

Summary

The centrosome is the main microtubule-organizing centre. It also organizes a local network of actin filaments. However, the precise function of the actin network at the centrosome is not well understood. Here, we show that increasing densities of actin filaments at the centrosome of lymphocytes are correlated with reduced amounts of microtubules. Furthermore, lymphocyte activation resulted in disassembly of centrosomal actin and an increase in microtubule number. To further investigate the direct crosstalk between actin and microtubules at the centrosome, we performed reconstitution assays based on (i) purified centrosomes and (ii) on the co-micropatterning of microtubule seeds and actin filaments. These two assays demonstrated that actin filaments constitute a physical barrier blocking elongation of nascent microtubules. Finally, we showed that cell adhesion and cell spreading lead to lower densities of centrosomal actin, thus resulting in higher microtubule growth. We therefore propose a novel mechanism, by which the number of centrosomal microtubules is regulated by cell adhesion and actin-network architecture.

Vasco Rodrigues, Philippe Benaroch (2019 Mar 23)
Macrophages hide HIV in the urethra.
Nature microbiology : 556-557 : DOI : 10.1038/s41564-019-0418-5

Summary

Jamecna D, Polidori DJ, Mesmin B, Dezi M, Lévy D, Bigay J, Antonny B (2019 Mar 22)
An intrinsically disordered region in OSBP acts as an entropic barrier to control protein dynamics and orientation at membrane contact sites
Developmental cell : DOI : doi.org/10.1016/j.devcel.2019.02.021

Summary

Lipid transfer proteins (LTPs) acting at membrane contact sites (MCS) between the ER and other organelles contain domains involved in heterotypic (e.g. ER to Golgi) membrane tethering as well as domains involved in lipid transfer. Here, we show that a long ≈ 90 aa intrinsically unfolded sequence at the N-terminus of oxysterol binding protein (OSBP) controls OSBP orientation and dynamics at MCS. This Gly-Pro-Ala-rich sequence, whose hydrodynamic radius is twice as that of folded domains, prevents the two PH domains of the OSBP dimer from homotypically tethering two Golgi-like membranes and considerably facilitates OSBP in-plane diffusion and recycling at MCS. Although quite distant in sequence, the N-terminus of OSBP-related protein-4 (ORP4) has similar effects. We propose that N-terminal sequences of low complexity in ORPs form an entropic barrier that restrains protein orientation, limits protein density and facilitates protein mobility in the narrow and crowded MCS environment.

Simon C*, Kusters R*, Caorsi V*, Allard A, Abou-Ghali M, Manzi J, Di Cicco A, Lévy D, Lenz M, Joanny J-F, Campillo C, Plastino J, Sens P*, Sykes C* (2019 Mar 18)
Actin dynamics drive cell-like membrane deformation
Nature Physics : DOI : 10.1038/s41567-019-0464-1

Summary

Hélène Salmon, Romain Remark, Sacha Gnjatic, Miriam Merad (2019 Mar 15)
Host tissue determinants of tumour immunity.
Nature Reviews Cancer : 215-227 : DOI : 10.1038/s41568-019-0125-9

Summary

Although common evolutionary principles drive the growth of cancer cells regardless of the tissue of origin, the microenvironment in which tumours arise substantially differs across various organ sites. Recent studies have established that, in addition to cell-intrinsic effects, tumour growth regulation also depends on local cues driven by tissue environmental factors. In this Review, we discuss how tissue-specific determinants might influence tumour development and argue that unravelling the tissue-specific contribution to tumour immunity should help the development of precise immunotherapeutic strategies for patients with cancer.

Aria Ronsmans, Maxime Wery, Ugo Szachnowski, Camille Gautier, Marc Descrimes, Evelyne Dubois, Antonin Morillon, Isabelle Georis (2019 Mar 1)
Transcription-dependent spreading of the Dal80 yeast GATA factor across the body of highly expressed genes.
PLoS genetics : e1007999 : DOI : 10.1371/journal.pgen.1007999

Summary

GATA transcription factors are highly conserved among eukaryotes and play roles in transcription of genes implicated in cancer progression and hematopoiesis. However, although their consensus binding sites have been well defined in vitro, the in vivo selectivity for recognition by GATA factors remains poorly characterized. Using ChIP-Seq, we identified the Dal80 GATA factor targets in yeast. Our data reveal Dal80 binding to a large set of promoters, sometimes independently of GATA sites, correlating with nitrogen- and/or Dal80-sensitive gene expression. Strikingly, Dal80 was also detected across the body of promoter-bound genes, correlating with high expression. Mechanistic single-gene experiments showed that Dal80 spreading across gene bodies requires active transcription. Consistently, Dal80 co-immunoprecipitated with the initiating and post-initiation forms of RNA Polymerase II. Our work suggests that GATA factors could play dual, synergistic roles during transcription initiation and post-initiation steps, promoting efficient remodeling of the gene expression program in response to environmental changes.

Paul D., Marchand A., Verga D., Bombard S., Teulade-Fichou M.P., Rosu F., Gabelica V. (2019 Feb 28)

Probing Ligand and Cation Binding Sites in G-Quadruplex Nucleic Acids by Mass Spectrometry and Electron Photodetachment Dissociation Sequencing

bioRxiv : Early view : DOI : 10.1101/563627

Summary

Mass spectrometry provides exquisite detail on ligand and cation binding stoichiometries with a DNA target. The next important step is to develop reliable methods to determine the cation and ligand binding sites in each complex separated by the mass spectrometer. To circumvent the caveat of ligand derivatization for cross-linking, which may alter the ligand binding mode, we explored a tandem mass spectrometry (MS/MS) method that does not require ligand derivatization, and is therefore also applicable to localize metal cations. By obtaining more negative charge states for the complexes using supercharging agents, and by creating radical ions by electron photodetachment, oligonucleotide bonds become weaker than the DNA-cation or DNA-ligand noncovalent bonds upon collision-induced dissociation of the radicals. This electron photodetachment (EPD) method allows to locate the binding regions of cations and ligands by top-down sequencing of the oligonucleotide target. The very potent G-quadruplex ligands 360A and PhenDC3 were found to replace a potassium cation and bind close to the central loop of 4-repeat human telomeric sequences.