Year of publication 2019

CAMPAGNE Antoine, LEE Ming-Kang, ZIELINSKI Dina, MICHAUD Audrey, LE CORRE Stéphanie, DINGLI Florent, CHEN Hong, SHAHIDIAN Lara Z, SERVANT Nicolas, LOEW Damarys, PASMANT Eric, PISTEL-VINAY Sophie, WASSEF Michel, MARGUERON Raphaël (2019 Jan 21)

BAP1 complex promotes transcription by opposing PRC1-mediated H2A ubiquitylation

Nature Communications : DOI : 10.1038/s41467-018-08255-x

Summary

In Drosophila, a complex consisting of Calypso and ASX catalyzes H2A deubiquitination and has been reported to act as part of the Polycomb machinery in transcriptional silencing. The mammalian homologs of these proteins (BAP1 and ASXL1/2/3, respectively), are frequently mutated in various cancer types, yet their precise functions remain unclear. Using an integrative approach based on isogenic cell lines generated with CRISPR/Cas9, we uncover an unanticipated role for BAP1 in gene activation. This function requires the assembly of an enzymatically active BAP1-associated core complex (BAP1.com) containing one of the redundant ASXL proteins. We investigate the mechanism underlying BAP1.com-mediated transcriptional regulation and show that it does not participate in Polycomb-mediated silencing. Instead, our results establish that the function of BAP1.com is to safeguard transcriptionally active genes against silencing by the Polycomb Repressive Complex 1.

Year of publication 2018

Żylicz Jan Jakub, Bousard Aurélie, Žumer Kristina, Dossin François, Mohammad Eusra, Teixiera da Rocha Simão, Schwalb Björn, Syx Laurène, Dingli Florent, Loew Damarys, Cramer Patrick, Heard Edith (2018 Dec 21)

The Implication of Early Chromatin Changes in X Chromosome Inactivation

Cell : 176 : 1-16 : DOI : 10.1016/j.cell.2018.11.041

Summary

During development, the precise relationships between transcription and chromatin modifications often remain unclear. We use the X chromosome inactivation (XCI) paradigm to explore the implication of chromatin changes in gene silencing. Using female mouse embryonic stem cells, we initiate XCI by inducing Xist and then monitor the temporal changes in transcription and chromatin by allele-specific profiling. This reveals histone deacetylation and H2AK119 ubiquitination as the earliest chromatin alterations during XCI. We show that HDAC3 is pre-bound on the X chromosome and that, upon Xist coating, its activity is required for efficient gene silencing. We also reveal that first PRC1-associated H2AK119Ub and then PRC2-associated H3K27me3 accumulate initially at large intergenic domains that can then spread into genes only in the context of histone deacetylation and gene silencing. Our results reveal the hierarchy of chromatin events during the initiation of XCI and identify key roles for chromatin in the early steps of transcriptional silencing.

Elie Hatem, Sandy Azzi, Nadine El Banna, Tiantian He, Amélie Heneman-Masurel, Laurence Vernis, Dorothée Baïlle, Vanessa Masson, Florent Dingli, Damarys Loew, Bruno Azzarone, Pierre Eid, Giuseppe Baldacci, Meng-Er Huang (2018 Nov 20)

Auranofin/Vitamin C: A Novel Drug Combination Targeting Triple-Negative Breast Cancer.

Journal of the National Cancer Institute : DOI : 10.1093/ije/djy149

Summary

Cancer cells from different origins exhibit various basal redox statuses and thus respond differently to intrinsic or extrinsic oxidative stress. These intricate characteristics condition the success of redox-based anticancer therapies that capitalize on the ability of reactive oxygen species to achieve selective and efficient cancer cell killing.

Forget Antoine, Martignetti Loredana, Puget Stéphanie, Calzone Laurence, Brabetz Sebastian, Picard Daniel, Montagud Arnau, Liva Stéphane, Sta Alexandre, Dingli Florent, Arras Guillaume, Rivera Jaime, Loew Damarys, Besnard Aurore, Lacombe Joëlle, Pagès Mélanie, Varlet Pascale, Dufour Christelle, Yu Hua, L. Mercier Audrey, Indersie Emilie, Chivet Anaïs, Leboucher Sophie, Sieber Laura, Beccaria Kevin, Gombert Michael, D. Meyer Frauke, Qin Nan, Bartl Jasmin, Chavez Lukas, Okonechnikov Konstantin, Sharma Tanvi, Thatikonda Venu, Bourdeaut Franck, Pouponnot Celio, Ramaswamy Vijay, Korshunov Andrey, Borkhardt Arndt, Reifenberger Guido, Poullet Patrick, D. Taylor Michael, Kool Marcel, M. Pfister Stefan, Kawauchi Daisuke, Barillot Emmanuel, Remke Marc, Ayrault Olivier (2018 Sep 10)

Aberrant ERBB4-SRC Signaling as a Hallmark of Group 4 Medulloblastoma Revealed by Integrative Phosphoproteomic Profiling

Cancer Cell : 34 : 379-395 : DOI : 10.1016/j.ccell.2018.08.002

Summary

The current consensus recognizes four main medulloblastoma subgroups (wingless, Sonic hedgehog, group 3 and group 4). While medulloblastoma subgroups have been characterized extensively at the (epi-)genomic and transcriptomic levels, the proteome and phosphoproteome landscape remain to be comprehensively elucidated. Using quantitative (phospho)-proteomics in primary human medulloblastomas, we unravel distinct posttranscriptional regulation leading to highly divergent oncogenic signaling and kinase activity profiles in groups 3 and 4 medulloblastomas. Specifically, proteomic and phosphoproteomic analyses identify aberrant ERBB4-SRC signaling in group 4. Hence, enforced expression of an activated SRC combined with p53 inactivation induces murine tumors that resemble group 4 medulloblastoma. Therefore, our integrative proteogenomics approach unveils an oncogenic pathway and potential therapeutic vulnerability in the most common medulloblastoma subgroup.

Nassrallah Amr, Rougée Martin, Bourbousse Clara, Drevensek Stephanie, Fonseca Sandra, Iniesto Elisa, Ait-Mohamed Ouardia, Deton-Cabanillas Anne-Flore, Zabulon Gerald, Ahmed Ikhlak, Stroebel David, Masson Vanessa, Lombard Berangere, Eeckhout Dominique, Gevaert Kris, Loew Damarys, Genovesio Auguste, Breyton Cecile, de Jaeger Geert, Bowler Chris, Rubio Vicente, Barneche Fredy (2018 Sep 7)

DET1-mediated degradation of a SAGA-like deubiquitination module controls H2Bub homeostasis

eLIFE : DOI : 10.7554/eLife.37892

Summary

DE-ETIOLATED 1 (DET1) is an evolutionarily conserved component of the ubiquitination machinery that mediates the destabilization of key regulators of cell differentiation and proliferation in multicellular organisms. In this study, we provide evidence from Arabidopsis that DET1 is essential for the regulation of histone H2B monoubiquitination (H2Bub) over most genes by controlling the stability of a deubiquitination module (DUBm). In contrast with yeast and metazoan DUB modules that are associated with the large SAGA complex, the Arabidopsis DUBm only comprises three proteins (hereafter named SGF11, ENY2 and UBP22) and appears to act independently as a major H2Bub deubiquitinase activity. Our study further unveils that DET1-DDB1-Associated-1 (DDA1) protein interacts with SGF11 *in vivo*, linking the DET1 complex to light-dependent ubiquitin-mediated proteolytic degradation of the DUBm. Collectively, these findings uncover a signaling path controlling DUBm availability, potentially adjusting H2Bub turnover capacity to the cell transcriptional status

Verweij Frederik J, Revenu Celine, Arras Guillaume, Dingli Florent, Loew Damarys, Follain Gautier, Allio Guillaume, Goetz Jacky G., Herbomel Philippe, Del Bene Filippo, Raposo Graça, van Niel Guillaume (2018 Jul 30)

Live tracking of inter-organ communication by endogenous exosomes in vivo

BioRxiv : DOI : 10.1101/380311

Summary

Laencina Laura, Dubois Violaine, Le Moigne Vincent , Viljoen Albertus, Majlessi Laleh, Pritchard Justin, Bernut Audrey, Piel Laura, Roux Anne-Laure, Gaillard Jean-Louis, Lombard Bérengère, Loew Damarys, Rubin Eric J., Brosch Roland, Kremer Laurent, Herrmann Jean-Louis and Girard-Misguich Fabienne (2018 Jan 17)

Identification of genes required for Mycobacterium abscessus growth in vivo with a prominent role of the ESX-4 locus

Proceedings of the National Academy of Sciences of the United States of America : 115 : E1002-E1011 : DOI : 10.1073/pnas.1713195115

Summary

The coevolution of mycobacteria and amoebae seems to have contributed to shaping the virulence of nontuberculous mycobacteria in macrophages. We identified a pool of genes essential for the intracellular survival of *Mycobacterium abscessus* inside amoebae and macrophages and discovered a hot spot of transposon insertions within the orthologous ESX-4 T7SS locus. We generated a mutant with the deletion of a structural key ESX component, $EccB_4$. We demonstrate rupture of the phagosomal membrane only in the presence of an intact $eccB_4$ gene. These results suggest an unanticipated role of ESX-4 T7SS in governing the intracellular behavior of a mycobacterium. Because *M. abscessus* lacks ESX-1, it is tempting to speculate that ESX-4 operates as a surrogate for ESX-1 in *M. tuberculosis*.

Year of publication 2017

Sara Bizzotto, Ana Uzquiano, Florent Dingli, Dmitry Ershov, Anne Houllier, Guillaume Arras, Mark Richards, Damarys Loew, Nicolas Minc, Alexandre Croquelois, Anne Houdusse, Fiona Francis (2017 Dec 13)

Eml1 loss impairs apical progenitor spindle length and soma shape in the developing cerebral cortex.

Scientific reports : 17308 : DOI : 10.1038/s41598-017-15253-4

Summary

The ventricular zone (VZ) of the developing cerebral cortex is a pseudostratified epithelium that contains progenitors undergoing precisely regulated divisions at its most apical side, the ventricular lining (VL). Mitotic perturbations can contribute to pathological mechanisms leading to cortical malformations. The HeCo mutant mouse exhibits subcortical band heterotopia (SBH), likely to be initiated by progenitor delamination from the VZ early during corticogenesis. The causes for this are however, currently unknown. Eml1, a microtubule (MT)-associated protein of the EMAP family, is impaired in these mice. We first show that MT dynamics are perturbed in mutant progenitor cells in vitro. These may influence interphase and mitotic MT mechanisms and indeed, centrosome and primary cilia were altered and spindles were found to be abnormally long in HeCo progenitors. Consistently, MT and spindle length regulators were identified in EML1 pulldowns from embryonic brain extracts. Finally, we found that mitotic cell shape is also abnormal in the mutant VZ. These previously unidentified VZ characteristics suggest altered cell constraints which may contribute to cell delamination.

Lucie Hebert, Dorine Bellanger, Chloé Guillas, Antoine Campagne, Florent Dingli, Damarys Loew, Alice Fievet, Virginie Jacquemin, Tatiana Popova, Didier Jean, Fatima Mechta-Grigoriou, Raphaël Margueron, Marc-Henri Stern (2017 Oct 27)

Modulating BAP1 expression affects ROS homeostasis, cell motility and mitochondrial function.
Oncotarget : 72513-72527 : DOI : 10.18632/oncotarget.19872

Summary

The tumor suppressor BAP1 associates with ASXL1/2 to form the core Polycomb complex PR-DUB, which catalyzes the removal of mono-ubiquitin from several substrates including histone H2A. This complex also mediates the poly-deubiquitination of HCFC1, OGT and PCG1-α, preventing them from proteasomal degradation. Surprisingly, considering its role in a Polycomb complex, no transcriptional signature was consistently found among BAP1-inactivated tumor types. It was hypothesized that BAP1 tumor suppressor activity could reside, at least in part, in stabilizing proteins through its poly-deubiquitinase activity. Quantitative mass spectrometry and gene expression arrays were used to investigate the consequences of BAP1 expression modulation in the NCI-H226 mesothelioma cell line. Analysis of differentially expressed proteins revealed enrichment in cytoskeleton organization, mitochondrial activity and ROS management, while gene expression analysis revealed enrichment in the epithelial-to-mesenchymal transition pathway. Functional assessments in BAP1 inactivated, BAP1 wild-type and BAP1 catalytically dead-expressing NCI-H226 and QR mesothelioma cell lines confirmed alteration of these pathways and demonstrated that BAP1 deubiquitinase activity was mandatory to maintain these phenotypes. Interestingly, monitoring intracellular ROS levels partly restored the morphology and the mitochondrial activity. Finally, the study suggests new tumorigenic and cellular functions of BAP1 and shows for the first time the interest of studying the proteome as readout of BAP1 inactivation.

Alexandros Glentis, Philipp Oertle, Pascale Mariani, Aleksandra Chikina, Fatima El Marjou, Youmna Attieh, Francois Zaccarini, Marick Lae, Damarys Loew, Florent Dingli, Philemon Sirven, Marie Schoumacher, Basile G Gurchenkov, Marija Plodinec, Danijela Matic Vignjevic (2017 Oct 15)

Cancer-associated fibroblasts induce metalloprotease-independent cancer cell invasion of the basement membrane.
Nature communications : 924 : DOI : 10.1038/s41467-017-00985-8

Summary

At the stage of carcinoma in situ, the basement membrane (BM) segregates tumor cells from the stroma. This barrier must be breached to allow dissemination of the tumor cells to adjacent tissues. Cancer cells can perforate the BM using proteolysis; however, whether stromal cells play a role in this process remains unknown. Here we show that an abundant stromal cell population, cancer-associated fibroblasts (CAFs), promote cancer cell invasion through the BM. CAFs facilitate the breaching of the BM in a matrix metalloproteinase-independent manner. Instead, CAFs pull, stretch, and soften the BM leading to the formation of gaps through which cancer cells can migrate. By exerting contractile forces, CAFs alter the organization and the physical properties of the BM, making it permissive for cancer cell

invasion. Blocking the ability of stromal cells to exert mechanical forces on the BM could therefore represent a new therapeutic strategy against aggressive tumors.Stromal cells play various roles in tumor establishment and metastasis. Here the authors, using an ex-vivo model, show that cancer-associated fibroblasts facilitate colon cancer cells invasion in a matrix metalloproteinase-independent manner, likely by pulling and stretching the basement membrane to form gaps.

Gheghiani Lilia , Loew Damarys, Lombard Bérangère, Mansfeld Jörg, Gavet Olivier (2017 Jun 6)
PLK1 Activation in Late G2 Sets Up Commitment to Mitosis
Cell Reports : 19 : 2060-2073 : DOI : 10.1016/j.celrep.2017.05.031

Summary

Commitment to mitosis must be tightly coordinated with DNA replication to preserve genome integrity. While we have previously established that the timely activation of CyclinB1-Cdk1 in late G2 triggers mitotic entry, the upstream regulatory mechanisms remain unclear. Here, we report that Polo-like kinase 1 (Plk1) is required for entry into mitosis during an unperturbed cell cycle and is rapidly activated shortly before CyclinB1-Cdk1. We determine that Plk1 associates with the Cdc25C1 phosphatase and induces its phosphorylation before mitotic entry. Plk1-dependent Cdc25C1 phosphosites are sufficient to promote mitotic entry, even when Plk1 activity is inhibited. Furthermore, we find that activation of Plk1 during G2 relies on CyclinA2-Cdk activity levels. Our findings thus elucidate a critical role for Plk1 in CyclinB1-Cdk1 activation and mitotic entry and outline how CyclinA2-Cdk, an S-promoting factor, poises cells for commitment to mitosis.

Guillaume Kellermann, Florent Dingli, Vanessa Masson, Daniel Dauzonne, Evelyne Ségal-Bendirdjian, Marie-Paule Teulade-Fichou, Damarys Loew, Sophie Bombard (2017 Mar 1)
Exploring the mechanism of inhibition of human telomerase by cysteine-reactive compounds.
FEBS letters : 591 : 863-874 : DOI : 10.1002/1873-3468.12589

Summary

Telomerase is an almost universal cancer target that consists minimally of a core protein (hTERT) and an RNA (hTR). Some inhibitors of this enzyme are thought to function by the covalent binding to one or several cystein residues; however, this inhibition mechanism has never been investigated because of the difficulty in producing telomerase. In the present study, we use a recent method to produce recombinant hTERT to analyse the effect of cysteine reactive inhibitors on telomerase. Using mass-spectrometry (MS) and mutagenesis analysis, we identify several targeted residues in separated domains of the hTERT protein and show that cysteine-reactive reagents abolish the interaction with the CR4/5 region of hTR.

Sergio A Rincon, Miguel Estravis, Florent Dingli, Damarys Loew, Phong T Tran, Anne Paoletti (2017 Feb 7)

SIN-Dependent Dissociation of the SAD Kinase Cdr2 from the Cell Cortex Resets the Division Plane.

Current biology : CB : 534-542 : DOI : 10.1016/j.cub.2016.12.050

Summary

Proper division plane positioning is crucial for faithful chromosome segregation but also influences cell size, position, or fate [1]. In fission yeast, medial division is controlled through negative signaling by the cell tips during interphase and positive signaling by the centrally placed nucleus at mitotic entry [2-4]: the cell geometry network (CGN), controlled by the inhibitory cortical gradient of the DYRK kinase Pom1 emanating from the cell tips, first promotes the medial localization of cytokinetic ring precursors organized by the SAD kinase Cdr2 to pre-define the division plane [5-8]; then, massive nuclear export of the anillin-like protein Mid1 at mitosis entry confirms or readjusts the division plane according to nuclear position and triggers the assembly of a medial contractile ring [5, 9-11]. Strikingly, the Hippo-like septation initiation network (SIN) induces Cdr2 dissociation from cytokinetic precursors at this stage [12-14]. We show here that SIN-dependent phosphorylation of Cdr2 promotes its interaction with the 14-3-3 protein Rad24 that sequesters it in the cytoplasm during cell division. If this interaction is compromised, cytokinetic precursors are asymmetrically distributed in the cortex of newborn cells, leading to asymmetrical division if nuclear signaling is abolished. We conclude that, through this new function, the SIN resets the division plane in newborn cells to ensure medial division.

Yann Duroc, Rajeev Kumar, Lepakshi Ranjha, Céline Adam, Raphaël Guérois, Khan Md Muntaz, Marie-Claude Marsolier-Kergoat, Florent Dingli, Raphaëlle Laureau, Damarys Loew, Bertrand Llorente, Jean-Baptiste Charbonnier, Petr Cejka, Valérie Borde (2017 Jan 5)

Concerted action of the MutLβ heterodimer and Mer3 helicase regulates the global extent of meiotic gene conversion.

eLife : DOI : 10.7554/eLife.21900

Summary

Gene conversions resulting from meiotic recombination are critical in shaping genome diversification and evolution. How the extent of gene conversions is regulated is unknown. Here we show that the budding yeast mismatch repair related MutLβ complex, Mlh1-Mlh2, specifically interacts with the conserved meiotic Mer3 helicase, which recruits it to recombination hotspots, independently of mismatch recognition. This recruitment is essential to limit gene conversion tract lengths genome-wide, without affecting crossover formation. Contrary to expectations, Mer3 helicase activity, proposed to extend the displacement loop (D-loop) recombination intermediate, does not influence the length of gene conversion events, revealing non-catalytical roles of Mer3. In addition, both purified Mer3 and MutLβ preferentially recognize D-loops, providing a mechanism for limiting gene conversion in vivo. These findings show that MutLβ is an integral part of a new regulatory step of meiotic

recombination, which has implications to prevent rapid allele fixation and hotspot erosion in populations.

Year of publication 2016

Cédric M Blouin, Yannick Hamon, Pauline Gonnord, Cédric Boularan, Jérémy Kagan, Christine Viaris de Lesegno, Richard Ruez, Sébastien Mailfert, Nicolas Bertaux, Damarys Loew, Christian Wunder, Ludger Johannes, Guillaume Vogt, Francesc-Xabier Contreras, Didier Marguet, Jean-Laurent Casanova, Céline Galès, Hai-Tao He, Christophe Lamaze (2016 Aug 9)

Glycosylation-Dependent IFN-γR Partitioning in Lipid and Actin Nanodomains Is Critical for JAK Activation.

Cell : 920-34 : DOI : 10.1016/j.cell.2016.07.003

Summary

Understanding how membrane nanoscale organization controls transmembrane receptors signaling activity remains a challenge. We studied interferon-γ receptor (IFN-γR) signaling in fibroblasts from homozygous patients with a T168N mutation in IFNGR2. By adding a neo-N-glycan on IFN-γR2 subunit, this mutation blocks IFN-γ activity by unknown mechanisms. We show that the lateral diffusion of IFN-γR2 is confined by sphingolipid/cholesterol nanodomains. In contrast, the IFN-γR2 T168N mutant diffusion is confined by distinct actin nanodomains where conformational changes required for Janus-activated tyrosine kinase/signal transducer and activator of transcription (JAK/STAT) activation by IFN-γ could not occur. Removing IFN-γR2 T168N-bound galectins restored lateral diffusion in lipid nanodomains and JAK/STAT signaling in patient cells, whereas adding galectins impaired these processes in control cells. These experiments prove the critical role of dynamic receptor interactions with actin and lipid nanodomains and reveal a new function for receptor glycosylation and galectins. Our study establishes the physiological relevance of membrane nanodomains in the control of transmembrane receptor signaling in vivo. VIDEO ABSTRACT.