Année de 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

Résumé

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.

Année de 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

Résumé

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

Résumé

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

Résumé

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

Résumé

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

Résumé

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

Résumé

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*.

Année de publication : 2017

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

Résumé

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.

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

Résumé

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

Résumé

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.

Année de publication : 2016

Dorian Obino, Francesca Farina, Odile Malbec, Pablo J Sáez, Mathieu Maurin, Jérémie Gaillard, Florent Dingli, Damarys Loew, Alexis Gautreau, Maria-Isabel Yuseff, Laurent Blanchoin, Manuel Théry, Ana-Maria Lennon-Duménil (2016 Mar 19)

Actin nucleation at the centrosome controls lymphocyte polarity

Nature communications : 10969 : DOI : 10.1038/ncomms10969

Résumé

Cell polarity is required for the functional specialization of many cell types including lymphocytes. A hallmark of cell polarity is the reorientation of the centrosome that allows repositioning of organelles and vesicles in an asymmetric fashion. The mechanisms underlying centrosome polarization are not fully understood. Here we found that in resting lymphocytes, centrosome-associated Arp2/3 locally nucleates F-actin, which is needed for centrosome tethering to the nucleus via the LINC complex. Upon lymphocyte activation, Arp2/3 is partially depleted from the centrosome as a result of its recruitment to the immune synapse. This leads to a reduction in F-actin nucleation at the centrosome and thereby allows its detachment from the nucleus and polarization to the synapse. Therefore, F-actin nucleation at the centrosome-regulated by the availability of the Arp2/3 complex-determines its capacity to polarize in response to external stimuli.

Joanna Kowal, Guillaume Arras, Marina Colombo, Mabel Jouve, Jakob Paul Morath, Bjarke Primdal-Bengtson, Florent Dingli, Damarys Loew, Mercedes Tkach, Clotilde Théry (2016 Feb 8)
Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes.
PNAS : 113: E968-977 : DOI : 10.1073/pnas.1521230113

Résumé

Extracellular vesicles (EVs) have become the focus of rising interest because of their numerous functions in physiology and pathology. Cells release heterogeneous vesicles of different sizes and intracellular origins, including small EVs formed inside endosomal compartments (i.e., exosomes) and EVs of various sizes budding from the plasma membrane. Specific markers for the analysis and isolation of different EV populations are missing, imposing important limitations to understanding EV functions. Here, EVs from human dendritic cells were first separated by their sedimentation speed, and then either by their behavior upon upward floatation into iodixanol gradients or by immuno-isolation. Extensive quantitative proteomic analysis allowing comparison of the isolated populations showed that several classically used exosome markers, like major histocompatibility complex, flotillin, and heat-shock 70-kDa proteins, are similarly present in all EVs. We identified proteins specifically enriched in small EVs, and define a set of five protein categories displaying different relative abundance in distinct EV populations. We demonstrate the presence of exosomal and nonexosomal subpopulations within small EVs, and propose their differential separation by immuno-isolation using either CD63, CD81, or CD9. Our work thus provides guidelines to define subtypes of EVs for future functional studies.

Année de publication : 2015

Anne Lafon, Surayya Taranum, Federico Pietrocola, Florent Dingli, Damarys Loew, Sandipan Brahma, Blaine Bartholomew, Manolis Papamichos-Chronakis (2015 Dec 15)

INO80 Chromatin Remodeler Facilitates Release of RNA Polymerase II from Chromatin for Ubiquitin-Mediated Proteasomal Degradation.

Molecular cell : 784-96 : DOI : 10.1016/j.molcel.2015.10.028

Résumé

Stalling of RNA Polymerase II (RNAPII) on chromatin during transcriptional stress results in polyubiquitination and degradation of the largest subunit of RNAPII, Rpb1, by the ubiquitin proteasome system (UPS). Here, we report that the ATP-dependent chromatin remodeling complex INO80 is required for turnover of chromatin-bound RNAPII in yeast. INO80 interacts physically and functionally with Cdc48/p97/VCP, a component of UPS required for degradation of RNAPII. Cells lacking INO80 are defective in Rpb1 degradation and accumulate tightly bound ubiquitinated Rpb1 on chromatin. INO80 forms a ternary complex with RNAPII and Cdc48 and targets Rpb1 primed for degradation. The function of INO80 in RNAPII turnover is required for cell growth and survival during genotoxic stress. Our results identify INO80 as a bona fide component of the proteolytic pathway for RNAPII degradation and suggest that INO80 nucleosome remodeling activity promotes the dissociation of ubiquitinated Rpb1 from chromatin to protect the integrity of the genome.

Aurelia Kuster, Sebastien Nola, Florent Dingli, Barbara Vacca, Christian Gauchy, Jean-Claude Beaujouan, Marcela Nunez, Thomas Moncion, Damarys Loew, Etienne Formstecher, Thierry Galli, Veronique Proux-Gillardeaux (2015 Sep 12)

The Q-soluble N-Ethylmaleimide-sensitive Factor Attachment Protein Receptor (Q-SNARE) SNAP-47 Regulates Trafficking of Selected Vesicle-associated Membrane Proteins (VAMPs).

The Journal of biological chemistry : 28056-69 : DOI : 10.1074/jbc.M115.666362

Résumé

SNAREs constitute the core machinery of intracellular membrane fusion, but vesicular SNAREs localize to specific compartments via largely unknown mechanisms. Here, we identified an interaction between VAMP7 and SNAP-47 using a proteomics approach. We found that SNAP-47 mainly localized to cytoplasm, the endoplasmic reticulum (ER), and ERGIC and could also shuttle between the cytoplasm and the nucleus. SNAP-47 preferentially interacted with the trans-Golgi network VAMP4 and post-Golgi VAMP7 and -8. SNAP-47 also interacted with ER and Golgi syntaxin 5 and with syntaxin 1 in the absence of Munc18a, when syntaxin 1 is retained in the ER. A C-terminally truncated SNAP-47 was impaired in interaction with VAMPs and affected their subcellular distribution. SNAP-47 silencing further shifted the subcellular localization of VAMP4 from the Golgi apparatus to the ER. WT and mutant SNAP-47 overexpression impaired VAMP7 exocytic activity. We conclude that SNAP-47 plays a role in the proper localization and function of a subset of VAMPs likely via regulation of their transport through the early secretory pathway.

Guillaume Kellermann, Markus Kaiser, Florent Dingli, Olivier Lahuna, Delphine Naud-Martin, Florence Mahuteau-Betzer, Damarys Loew, Evelyne Ségal-Bendirdjian, Marie-Paule Teulade-Fichou, Sophie Bombard (2015 Sep 3)

Identification of human telomerase assembly inhibitors enabled by a novel method to produce hTERT.

Nucleic acids research : 43 : e99 : DOI : 10.1093/nar/gkv425

Résumé

Telomerase is the enzyme that maintains the length of telomeres. It is minimally constituted of two components: a core reverse transcriptase protein (hTERT) and an RNA (hTR). Despite its significance as an almost universal cancer target, the understanding of the structure of telomerase and the optimization of specific inhibitors have been hampered by the limited amount of enzyme available. Here, we present a breakthrough method to produce unprecedented amounts of recombinant hTERT and to reconstitute human telomerase with purified components. This system provides a decisive tool to identify regulators of the assembly of this ribonucleoprotein complex. It also enables the large-scale screening of small-molecules capable to interfere with telomerase assembly. Indeed, it has allowed us to identify a compound that inhibits telomerase activity when added prior to the assembly of the enzyme, while it has no effect on an already assembled telomerase. Therefore, the novel system presented here may accelerate the understanding of human telomerase assembly and facilitate the discovery of potent and mechanistically unique inhibitors.