Année de publication : 2021

Guilherme Pedreira de Freitas Nader, Sonia Agüera-Gonzalez, Fiona Routet, Matthieu Gratia, Mathieu Maurin, Valeria Cancila, Clotilde Cadart, Andrea Palamidessi, Rodrigo Nalio Ramos, Mabel San Roman, Matteo Gentili, Ayako Yamada, Alice Williart, Catalina Lodillinsky, Emilie Lagoutte, Catherine Villard, Jean-Louis Viovy, Claudio Tripodo, Jérôme Galon, Giorgio Scita, Nicolas Manel, Philippe Chavrier, Matthieu Piel (2021 Sep 22)

Compromised nuclear envelope integrity drives TREX1-dependent DNA damage and tumor cell invasion.

Cell : DOI : S0092-8674(21)01046-1

Résumé

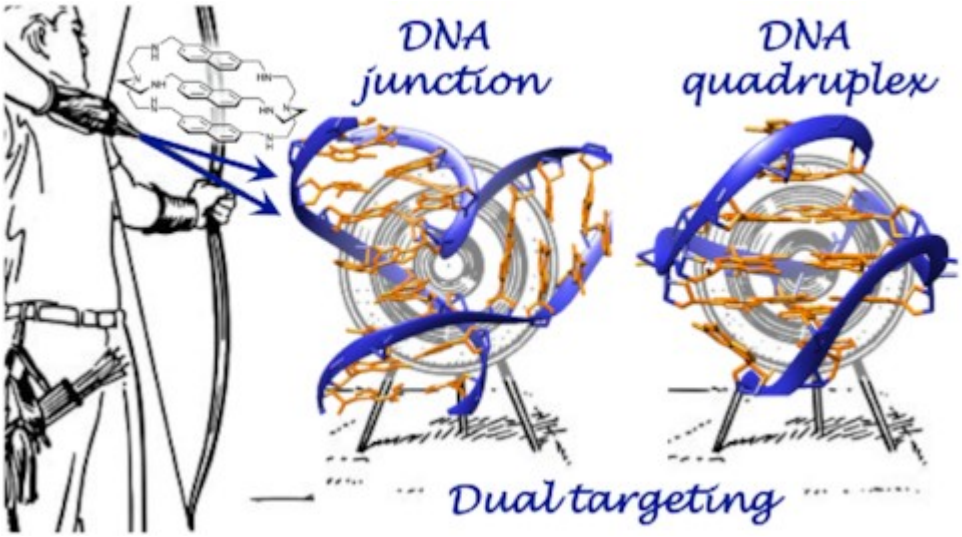
Although mutations leading to a compromised nuclear envelope cause diseases such as muscular dystrophies or accelerated aging, the consequences of mechanically induced nuclear envelope ruptures are less known. Here, we show that nuclear envelope ruptures induce DNA damage that promotes senescence in non-transformed cells and induces an invasive phenotype in human breast cancer cells. We find that the endoplasmic reticulum (ER)-associated exonuclease TREX1 translocates into the nucleus after nuclear envelope rupture and is required to induce DNA damage. Inside the mammary duct, cellular crowding leads to nuclear envelope ruptures that generate TREX1-dependent DNA damage, thereby driving the progression of in situ carcinoma to the invasive stage. DNA damage and nuclear envelope rupture markers were also enriched at the invasive edge of human tumors. We propose that DNA damage in mechanically challenged nuclei could affect the pathophysiology of crowded tissues by modulating proliferation and extracellular matrix degradation of normal and transformed cells.

Joanna Zell, Katerina Duskova, Leïla Chouh, Madeleine Bossaert, Nicolas Chéron, Anton Granzhan, Sébastien Britton, David Monchaud (2021 Sep 22)

Dual targeting of higher-order DNA structures by azacryptands induces DNA junction-mediated DNA damage in cancer cells

Nucleic Acids Research : 49 : 10275–10288 : DOI : 10.1093/nar/gkab796

Résumé



DNA is intrinsically dynamic and folds transiently into alternative higher-order structures such as G-quadruplexes (G4s) and three-way DNA junctions (TWJs). G4s and TWJs can be stabilised by small molecules (ligands) that have high chemotherapeutic potential, either as standalone DNA damaging agents or combined in synthetic lethality strategies. While previous approaches have claimed to use ligands that specifically target either G4s or TWJs, we report here on a new approach in which ligands targeting both TWJs and G4s *in vitro* demonstrate cellular effects distinct from that of G4 ligands, and attributable to TWJ targeting. The DNA binding modes of these new, dual TWJ-/G4-ligands were studied by a panel of *in vitro* methods and theoretical simulations, and their cellular properties by extensive cell-based assays. We show here that cytotoxic activity of TWJ-/G4-ligands is mitigated by the DNA damage response (DDR) and DNA topoisomerase 2 (TOP2), making them different from typical G4-ligands, and implying a pivotal role of TWJs in cells. We designed and used a clickable ligand, TrisNP-α, to provide unique insights into the TWJ landscape in cells and its modulation upon co-treatments. This wealth of data was exploited to design an efficient synthetic lethality strategy combining dual ligands with clinically relevant DDR inhibitors.

Aleksandr S. Oshchepkov, Oksana Reznichenko, Dan Xu, Boris S. Morozov, Anton Granzhan, Evgeny A. Kataev (2021 Sep 22)

Dye-functionalized Phosphate-binding Macrocycles: From Nucleotide to G-quadruplex Recognition and "turn-on" Fluorescence Sensing

Chemical Communications : Accepted Manuscript : DOI : 10.1039/D1CC04096K

Résumé

A novel strategy to design "turn-on" fluorescent receptors for G-quadruplexes of DNA is presented, which relies on the connection of phosphate binding macrocycles (PBM) with naphthalimide dyes. A new PBM-dye family was synthesized and evaluated in terms of binding and detection of nucleotides and DNA G-quadruplexes of different topologies.

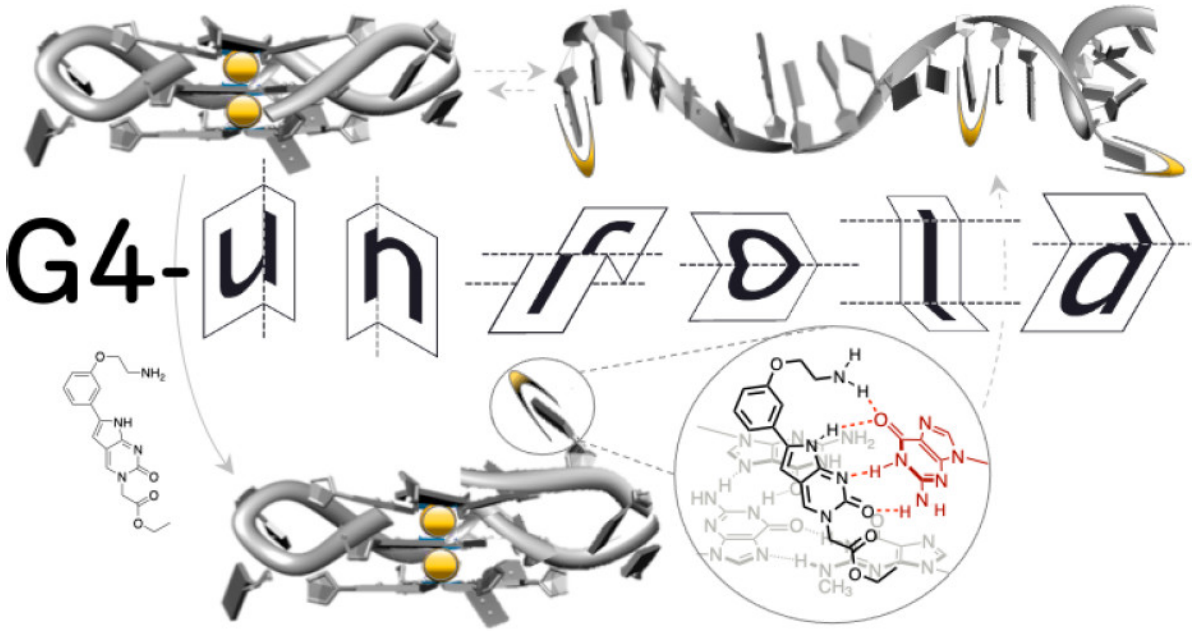
Jérémie Mitteaux, Pauline Lejault, Filip Wojciechowski, Alexandra Joubert, Julien Boudon, Nicolas Desbois, Claude P. Gros, Robert H. E. Hudson, Jean-Baptiste Boulé, Anton Granzhan, David Monchaud (2021 Aug 4)

Identifying G-Quadruplex-DNA-Disrupting Small Molecules

Journal of the American Chemical Society : 143 : 12567–12577 : DOI : 10.1021/jacs.1c04426

Résumé

The quest for small molecules that strongly bind to G-quadruplex-DNA (G4), so-called G4 ligands, has invigorated the G4 research field from its very inception. Massive efforts have been invested to discover or rationally design G4 ligands, evaluate their G4-interacting properties in vitro through a series of now widely accepted and routinely implemented assays, and use them as innovative chemical biology tools to interrogate cellular networks that might involve G4s. In sharp contrast, only uncoordinated efforts aimed at developing small molecules that destabilize G4s have been invested to date, even though it is now recognized that such molecular tools would have tremendous application in neurobiology as many genetic and age-related diseases are caused by an overrepresentation of G4s. Herein, we report on our efforts to develop in vitro assays to reliably identify molecules able to destabilize G4s. This workflow comprises the newly designed G4-unfold assay, adapted from the G4-helicase assay implemented with Pif1, as well as a series of biophysical and biochemical techniques classically used to study G4/ligand interactions (CD, UV–vis, PAGE, and FRET-melting), and a qPCR stop assay, adapted from a *Taq*-based protocol recently used to identify G4s in the genomic DNA of *Schizosaccharomyces pombe*. This unique, multipronged approach leads to the characterization of a phenylpyrrolocytosine (PhpC)-based G-clamp analog as a prototype of G4-disrupting small molecule whose properties are validated through many different and complementary in vitro evaluations.



Deborah Bourc'his (2021 Jul 30)
Metastable epialleles are stable in their instability.
Nature genetics : 1121-1123 : DOI : 10.1038/s41588-021-00907-x

Résumé

Jihene Klibi, Claudine Joseph, Marc Delord, Aurelie Teissandier, Bruno Lucas, Christine Chomienne, Antoine Toubert, Deborah Bourc'his, Fabien Guidez, Kamel Benlagha (2021 Jul 20)
PLZF Acetylation Levels Regulate NKT Cell Differentiation.
Journal of immunology (Baltimore, Md. : 1950) : 809-823 : DOI : 10.4049/jimmunol.2001444

Résumé

The transcription factor promyelocytic leukemia zinc finger (PLZF) is encoded by the BTB domain-containing 16 () gene. Its repressor function regulates specific transcriptional programs. During the development of invariant NKT cells, PLZF is expressed and directs their effector program, but the detailed mechanisms underlying PLZF regulation of multistage NKT cell developmental program are not well understood. This study investigated the role of acetylation-induced PLZF activation on NKT cell development by analyzing mice expressing a mutant form of PLZF mimicking constitutive acetylation (PLZF) mice. NKT populations in PLZF mice were reduced in proportion and numbers of cells, and the cells present were blocked at the transition from developmental stage 1 to stage 2. NKT cell subset differentiation was also altered, with T-bet NKT1 and RORγt NKT17 subsets dramatically reduced and the emergence of a T-betRORγt NKT cell subset with features of cells in early developmental stages rather than mature NKT2 cells. Preliminary analysis of DNA methylation patterns suggested that activated PLZF acts on the DNA methylation signature to regulate NKT cells' entry into the early stages of development while repressing maturation. In wild-type NKT cells, deacetylation of PLZF is possible, allowing subsequent NKT cell differentiation. Interestingly, development of other innate lymphoid and myeloid cells that are dependent on PLZF for their generation is not altered in PLZF mice, highlighting lineage-specific regulation. Overall, we propose that specific epigenetic control of PLZF through acetylation levels is required to regulate normal NKT cell differentiation.

Marc Lavigne, Olivier Helynck, Pascal Rigolet, Rofia Boudria-Souilah, Mireille Nowakowski, Bruno Baron, Sébastien Brülé, Sylviane Hoos, Bertrand Raynal, Lionel Guittat, Claire Beauvineau, Stéphane Petres, Anton Granzhan, Jean Guillon, Geneviève Pratviel, Marie-Paule Teulade-Fichou, Patrick England, Jean-Louis Mergny, Hélène Munier-Lehmann (2021 Jul 7)
SARS-CoV-2 Nsp3 unique domain SUD interacts with guanine quadruplexes and G4-ligands inhibit this interaction.
Nucleic Acids Research : 49 : 7695–7712 : DOI : 10.1093/nar/gkab571

Résumé

The multidomain non-structural protein 3 (Nsp3) is the largest protein encoded by coronavirus (CoV) genomes and several regions of this protein are essential for viral replication. Of note, SARS-CoV Nsp3 contains a SARS-Unique Domain (SUD), which can bind Guanine-rich non-canonical nucleic acid structures called G-quadruplexes (G4) and is essential for SARS-CoV replication. We show herein that the SARS-CoV-2 Nsp3 protein also contains a SUD domain that interacts with G4s. Indeed, interactions between SUD proteins and both DNA and RNA G4s were evidenced by G4 pull-down, Surface Plasmon Resonance and Homogenous Time Resolved Fluorescence. These interactions can be disrupted by mutations that prevent oligonucleotides from folding into G4 structures and, interestingly, by molecules known as specific ligands of these G4s. Structural models for these interactions are proposed and reveal significant differences with the crystallographic and modeled 3D structures of the SARS-CoV SUD-NM/G4 interaction. Altogether, our results pave the way for further studies on the role of SUD/G4 interactions during SARS-CoV-2 replication and the use of inhibitors of these interactions as potential antiviral compounds.

V. Kapoor, C. Carabaña (2021 Jul 6)
Cell Tracking in 3D using deep learning segmentations
scipy

Résumé

Live-cell imaging is a highly used technique to study cell migration and dynamics over time. Although many computational tools have been developed during the past years to automatically detect and track cells, they are optimized to detect cell nuclei with similar shapes and/or cells not clustering together. These existing tools are challenged when tracking fluorescently labelled membranes of cells due to cell's irregular shape, variability in size and dynamic movement across Z planes making it difficult to detect and track them. Here we introduce a detailed analysis pipeline to perform segmentation with accurate shape information, combined with BTrackmate, a customized codebase of popular ImageJ/Fiji software Trackmate, to perform cell tracking inside the tissue of interest. We developed VollSeg, a new segmentation method able to detect membrane-labelled cells with low signal-to-noise ratio and dense packing. Finally, we also created an interface in Napari, an Euler angle based viewer, to visualize the tracks along a chosen view making it possible to follow a cell along the plane of motion. Importantly, we provide a detailed protocol to implement this pipeline in a new dataset, together with the required Jupyter notebooks.

Valentin Laplaud, Nicolas Levernier, Judith Pineau, Mabel San Roman, Lucie Barbier, Pablo J Sáez, Ana-Maria Lennon-Duménil, Pablo Vargas, Karsten Kruse, Olivia du Roure, Matthieu Piel, Julien Heuvingh (2021 Jul 3)
Pinching the cortex of live cells reveals thickness instabilities caused by myosin II motors.
Science advances : DOI : eabe3640

Résumé

The cell cortex is a contractile actin meshwork, which determines cell shape and is essential for cell mechanics, migration, and division. Because its thickness is below optical resolution, there is a tendency to consider the cortex as a thin uniform two-dimensional layer. Using two mutually attracted magnetic beads, one inside the cell and the other in the extracellular medium, we pinch the cortex of dendritic cells and provide an accurate and time-resolved measure of its thickness. Our observations draw a new picture of the cell cortex as a highly dynamic layer, harboring large fluctuations in its third dimension because of actomyosin contractility. We propose that the cortex dynamics might be responsible for the fast shape-changing capacity of highly contractile cells that use amoeboid-like migration.

Emmanuelle Jeannot, Aurélien Latouche, Claire Bonneau, Marie-Ange Calméjane, Corine M Beaufort, Kirsten Ruigrok-Ritstier, Guillaume Bataillon, Linda Larbi Chérif, Celia Dupain, Charlotte Lecerf, Marina Popovic, Anne de la Rochefordière, Fabrice Lecuru, Virginie Fourchotte, Ekaterina S Jordanova, Heiko von der Leyen, Carine Tran-Perennou, Marie-Emmanuelle Legrier, Sylvain Dureau, Laurence Raizonville, Diana Bello Roufai, Christophe Le Tourneau, Ivan Bieche, Roman Rouzier, Els M J J Berns, Maud Kamal, Suzy Scholl (2021 Jul 2)

Circulating HPV DNA as a marker for early detection of relapse in patients with cervical cancer.

Clinical cancer research : an official journal of the American Association for Cancer Research : DOI : clincanres.0625.2021

Résumé

Almost all cervical cancers (CC) are caused by human papillomavirus (HPV) and patients with advanced stage are at high risk for relapse. Circulating HPV DNA (HPV ctDNA) may serve as a residual tumor marker at the end of chemo-radiation or to predict relapse during the follow-up period.

M. Plays, S. Müller, R. Rodriguez (2021 Jul 1)

Chemistry and Biology of Ferritin

Metallomics : DOI : 10.1093/mtomcs/mfab021

Résumé

Piguel S., Le Bescont J., Mouawad L., Boddaert T., Bombard S. (2021 Jun 29)

Photoactivatable small-molecule inhibitors for light-controlled TAM kinase activity

ChemPhotoChem : Accepted Author Manuscript : DOI : 10.1002/cptc.202100131

Résumé

The TAM kinase family arises as a promising therapeutical target for cancer therapy, auto-immune, and viral diseases. In this study, we report the first photoactivatable caged inhibitors of Tyro3 and Mer. This strategy enables spatial and temporal control of the biological activity of the inhibitor upon irradiation with UV light. We describe the design, the synthesis, the photocleavage properties, and the inhibitory activity of four Tyro3 and Mer photoactivatable small molecules. The proof of concept on the TAM kinase family was achieved in vitro , since irradiation by UV light restored the full inhibitory activity of two prodrugs.

Jakub Muraszko, Karol Kramarz, Bilge Argunhan, Kentaro Ito, Gabriela Baranowska, Yumiko Kurokawa, Yasuto Murayama, Hideo Tsubouchi, Sarah Lambert, Hiroshi Iwasaki, Dorota Dziadkowiec (2021 Jun 22)

Rrp1 translocase and ubiquitin ligase activities restrict the genome destabilising effects of Rad51 in fission yeast.

Nucleic acids research : DOI : gkab511

Résumé

Rad51 is the key protein in homologous recombination that plays important roles during DNA replication and repair. Auxiliary factors regulate Rad51 activity to facilitate productive recombination, and prevent inappropriate, untimely or excessive events, which could lead to genome instability. Previous genetic analyses identified a function for Rrp1 (a member of the Rad5/16-like group of SWI2/SNF2 translocases) in modulating Rad51 function, shared with the Rad51 mediator Swi5-Sfr1 and the Srs2 anti-recombinase. Here, we show that Rrp1 overproduction alleviates the toxicity associated with excessive Rad51 levels in a manner dependent on Rrp1 ATPase domain. Purified Rrp1 binds to DNA and has a DNA-dependent ATPase activity. Importantly, Rrp1 directly interacts with Rad51 and removes it from double-stranded DNA, confirming that Rrp1 is a translocase capable of modulating Rad51 function. Rrp1 affects Rad51 binding at centromeres. Additionally, we demonstrate in vivo and in vitro that Rrp1 possesses E3 ubiquitin ligase activity with Rad51 as a substrate, suggesting that Rrp1 regulates Rad51 in a multi-tiered fashion.

Katrina Cristall, Francois-Clement Bidard, Jean-Yves Pierga, Michael J Rauh, Tatiana Popova, Clara Sebbag, Olivier Lantz, Marc-Henri Stern, Christopher R Mueller (2021 Jun 17)

A DNA methylation-based liquid biopsy for triple-negative breast cancer.

NPJ precision oncology : 53 : DOI : 10.1038/s41698-021-00198-9

Résumé

Here, we present a next-generation sequencing (NGS) methylation-based blood test called methylation DETEction of Circulating Tumour DNA (mDETECT) designed for the optimal

detection and monitoring of metastatic triple-negative breast cancer (TNBC). Based on a highly multiplexed targeted sequencing approach, this assay incorporates features that offer superior performance and included 53 amplicons from 47 regions. Analysis of a previously characterised cohort of women with metastatic TNBC with limited quantities of plasma (<2 ml) produced an AUC of 0.92 for detection of a tumour with a sensitivity of 76% for a specificity of 100%. mDETECT was quantitative and showed superior performance to an NGS TP53 mutation-based test carried out on the same patients and to the conventional CA15-3 biomarker. mDETECT also functioned well in serum samples from metastatic TNBC patients where it produced an AUC of 0.97 for detection of a tumour with a sensitivity of 93% for a specificity of 100%. An assay for BRCA1 promoter methylation was also incorporated into the mDETECT assay and functioned well but its clinical significance is currently unclear. Clonal Hematopoiesis of Indeterminate Potential was investigated as a source of background in control subjects but was not seen to be significant, though a link to adiposity may be relevant. The mDETECT assay is a liquid biopsy able to quantitatively detect all TNBC cancers and has the potential to improve the management of patients with this disease.

Guillaume Jacquemin, Maria Benavente-Diaz, Samir Djaber, Aurélien Bore, Virginie Dangles-Marie, Didier Surdez, Shahragim Tajbakhsh, Silvia Fre, Bethan Lloyd-Lewis (2021 Jun 17)
Longitudinal high-resolution imaging through a flexible intravital imaging window.
Science advances : DOI : eabg7663

Résumé

Intravital microscopy (IVM) is a powerful technique that enables imaging of internal tissues at (sub)cellular resolutions in living animals. Here, we present a silicone-based imaging window consisting of a fully flexible, sutureless design that is ideally suited for long-term, longitudinal IVM of growing tissues and tumors. Crucially, we show that this window, without any customization, is suitable for numerous anatomical locations in mice using a rapid and standardized implantation procedure. This low-cost device represents a substantial technological and performance advance that facilitates intravital imaging in diverse contexts in higher organisms, opening previously unattainable avenues for in vivo imaging of soft and fragile tissues.