Année de publication : 2021

Judith Miné-Hattab, Mathias Heltberg, Marie Villemeur, Chloé Guedj, Thierry Mora, Aleksandra M Walczak, Maxime Dahan, Angela Taddei (2021 Feb 5)

Single molecule microscopy reveals key physical features of repair foci in living cells.

eLife : DOI : 10.7554/eLife.60577

Résumé

In response to double strand breaks (DSB), repair proteins accumulate at damaged sites, forming membrane-less sub-compartments or foci. Here we explored the physical nature of these foci, using single molecule microscopy in living cells. Rad52, the functional homolog of BRCA2 in yeast, accumulates at DSB sites and diffuses ~6 times faster within repair foci than the focus itself, exhibiting confined motion. The Rad52 confinement radius coincides with the focus size: foci resulting from 2 DSBs are twice larger in volume that the ones induced by a unique DSB and the Rad52 confinement radius scales accordingly. In contrast, molecules of the single strand binding protein Rfa1 follow anomalous diffusion similar to the focus itself or damaged chromatin. We conclude that while most Rfa1 molecules are bound to the ssDNA, Rad52 molecules are free to explore the entire focus reflecting the existence of a liquid droplet around damaged DNA.

Thomas Barbot, Veronica Beswick, Cédric Montigny, Éric Quiniou, Nadège Jamin and Liliane Mouawad (2021 Feb 4)

Deciphering the mechanism of inhibition of SERCA1a by sarcolipin using molecular simulations

Frontiers in Molecular Biosciences : 7 : 606254 : DOI : 10.3389/fmolb.2020.606254

Résumé

SERCA1a is an ATPase calcium pump that transports Ca2+ from the cytoplasm to the sarco/endoplasmic reticulum lumen. Sarcolipin (SLN), a transmembrane peptide, regulates the activity of SERCA1a by decreasing its Ca2+ transport rate, but its mechanism of action is still not well understood. To decipher this mechanism, we have performed normal mode analysis in the all-atom model, with the SERCA1a-SLN complex, or the isolated SERCA1a, embedded in an explicit membrane. The comparison of the results allowed us to provide an explanation at the atomic level for the action of SLN that is in good agreement with experimental observations. In our analyses, the presence of SLN locally perturbs the TM6 transmembrane helix and as a consequence modifies the position of D800, one of the key metal-chelating residues. Additionally, it reduces the flexibility of the gating residues, V304 and E309 in TM4, at the entrance of the Ca2+ binding sites, which would decrease the affinity for Ca2+. Unexpectedly, SLN has also an effect on the ATP binding site more than 35 Å away, due to the straightening of TM5, a long helix considered as the spine of the protein. The straightening of TM5 modifies the structure of the P-N linker that sits above it, and which comprises the 351DKTG354 conserved motif, resulting in an increase of the distance

between ATP and the phosphorylation site. As a consequence, the turn-over rate could be affected. All this gives SERCA1a the propensity to go toward a Ca2+ low-affinity E2-like state in the presence of SLN and toward a Ca2+ high-affinity E1-like state in the absence of SLN. In addition to a general mechanism of inhibition of SERCA1a regulatory peptides, this study also provides an insight into the conformational transition between the E2 and E1 states.

Thérèse Truong, Fabienne Lesueur, Pierre-Emmanuel Sugier, Julie Guibon, Constance Xhaard, Mojgan Karimi, Om Kulkarni, Elise A Lucotte, Delphine Bacq-Daian, Anne Boland-Auge, Claire Mulot, Pierre Laurent-Puig, Claire Schvartz, Anne-Valérie Guizard, Yan Ren, Elisabeth Adjadj, Frédérique Rachédi, Francoise Borson-Chazot, Rosa Maria Ortiz, Juan J Lence-Anta, Celia María Pereda, Daniel F Comiskey, Huiling He, Sandya Liyanarachchi, Albert de la Chapelle, Rossella Elisei, Federica Gemignani, Hauke Thomsen, Asta Forsti, Anthony F Herzig, Anne-Louise Leutenegger, Carole Rubino, Evgenia Ostroumova, Ausrele Kesminiene, Marie-Christine Boutron-Ruault, Jean-François Deleuze, Pascal Guénel, Florent de Vathaire (2021 Feb 2)
Multiethnic genome-wide association study of differentiated thyroid cancer in the EPITHYR consortium.
International journal of cancer : DOI : 10.1002/ijc.33488

Résumé

Incidence of differentiated thyroid carcinoma (DTC) varies considerably between ethnic groups, with particularly high incidence rates in Pacific Islanders. DTC is one of the cancers with the highest familial risk suggesting a major role of genetic risk factors, but only few susceptibility loci were identified so far. In order to assess the contribution of known DTC susceptibility loci and to identify new ones, we conducted a multiethnic genome-wide association study (GWAS) in individuals of European ancestry and of Oceanian ancestry from Pacific Islands. Our study included 1554 cases/1973 controls of European ancestry and 301 cases/348 controls of Oceanian ancestry from seven population-based case-control studies participating to the EPITHYR consortium. All participants were genotyped using the OncoArray-500K Beadchip (Illumina). We confirmed the association with the known DTC susceptibility loci at 2q35, 8p12, 9q22.33 and 14q13.3 in the European ancestry population and suggested two novel signals at 1p31.3 and 16q23.2, which were associated with thyroid-stimulating hormone levels in previous GWAS. We additionally replicated an association with 5p15.33 reported previously in Chinese and European populations. Except at 1p31.3, all associations were in the same direction in the population of Oceanian ancestry. We also observed that the frequencies of risk alleles at 2q35, 5p15.33 and 16q23.2 were significantly higher in Oceanians than in Europeans. However, additional GWAS and epidemiological studies in Oceanian populations are needed to fully understand the highest incidence observed in these populations.

Paul Gueguen, Christina Metoikidou, Thomas Dupic, Myriam Lawand, Christel Goudot, Sylvain Baulande, Sonia Lameiras, Olivier Lantz, Nicolas Girard, Agathe Seguin-Givelet, Marine Lefevre,

Thierry Mora, Aleksandra M Walczak, Joshua J Waterfall, Sebastian Amigorena (2021 Jan 30)
Contribution of resident and circulating precursors to tumor-infiltrating CD8 T cell populations in lung cancer.
Science immunology : DOI : eabd5778

Résumé

Tumor-infiltrating lymphocytes (TILs), in general, and especially CD8 TILs, represent a favorable prognostic factor in non-small cell lung cancer (NSCLC). The tissue origin, regenerative capacities, and differentiation pathways of TIL subpopulations remain poorly understood. Using a combination of single-cell RNA and T cell receptor (TCR) sequencing, we investigate the functional organization of TIL populations in primary NSCLC. We identify two CD8 TIL subpopulations expressing memory-like gene modules: one is also present in blood (circulating precursors) and the other one in juxtatumor tissue (tissue-resident precursors). In tumors, these two precursor populations converge through a unique transitional state into terminally differentiated cells, often referred to as dysfunctional or exhausted. Differentiation is associated with TCR expansion, and transition from precursor to late-differentiated states correlates with intratumor T cell cycling. These results provide a coherent working model for TIL origin, ontogeny, and functional organization in primary NSCLC.

Marios Sotiropoulos, Elise Brisebard, Marine Le Dudal, Gregory Jouvion, Marjorie Juchaux, Delphine Crépin, Catherine Sebrie, Laurene Jourdain, Dalila Labiod, Charlotte Lamirault, Frederic Pouzoulet, Yolanda Prezado (2021 Jan 29)
X-rays minibeam radiation therapy at a conventional irradiator: Pilot evaluation in F98-glioma bearing rats and dose calculations in a human phantom.
Clinical and translational radiation oncology : 44-49 : DOI : 10.1016/j.ctro.2021.01.001

Résumé

Minibeam radiation therapy (MBRT) is a type of spatial fractionated radiotherapy that uses submillimetric beams. This work reports on a pilot study on normal tissue response and the increase of the lifespan of glioma-bearing rats when irradiated with a tabletop x-ray system. Our results show a significant widening of the therapeutic window for brain tumours treated with MBRT: an important proportion of long-term survivals (60%) coupled with a significant reduction of toxicity when compared with conventional (broad beam) irradiations. In addition, the clinical translation of the minibeam treatment at a conventional irradiator is evaluated through a possible human head treatment plan.

Anke Steinmetz, Thomas Yvorra, Pascal Retailleau, Olivier Lantz, Frédéric Schmidt (2021 Jan 28)
Datasets and analyses of molecular dynamics simulations of covalent binary and ternary complexes of MHC class I-related molecule/T-cell receptor (MR1/TCR) agonists to understand complex formation and conditions of

fluorescent labelling.
Data in brief : 106704 : DOI : 10.1016/j.dib.2020.106704

Résumé

Data of molecular dynamics (MD) simulations were obtained for mucosal-associated invariant T (MAIT) cell ligands complexed with MR1 or MR1/TCR. Ligands included in the simulations were natural ligands 5-(2-oxoethylideneamino)-6-D-ribitylaminouracil (5-OE-RU), 5-(2-oxopropylideneamino)-6-(D-ribitylamino)uracil (5-OP-RU), their C5′ ethinylated analogs in S or R configuration, as well as the corresponding fluorophore-reacted products. All-atom models of the binary and ternary complexes were constructed using PDB entry 4NQE and docked poses [1]. Missing loops, N- and C-termini were completed by homology modelling, the loop conformations optimized, and the models energy minimized prior to setup for MD simulations. A standard pre-equilibration protocol was applied before the production phase of 120 ns simulation as NPT ensemble at 300 K and 1 atm applying an explicit solvent model with OPLS3 force field parameters. Atomic coordinates and energies were recorded every 60 ps and 12 ps, respectively. The corresponding raw data files of the MD simulations are part of this dataset. All simulations were analysed with respect to root mean square deviations (rmsd) and root mean square fluctuations (rmsf) of the coordinates of protein and ligand atoms, stability of protein secondary structure, protein-ligand contacts, ligand torsion profiles, and ligand properties. More detailed statistics of non-covalent interaction counts were also collected. Radial distribution functions (rdf) were calculated when relevant. Visualization of the trajectories permits appreciation of the molecular dynamics of both, ligands and proteins and their interactions, thereby supporting drug design of MAIT cell ligands; furthermore, additional analysis of e.g. conformational changes or interactions not reported in the primary publication [1] can be performed on the data. The raw data may also be used as starting point for extension of the simulations or more sophisticated MD techniques.

Lieske H Schrijver, Antonis C Antoniou, Håkan Olsson, Thea M Mooij, Marie-José Roos-Blom, Leyla Azarang, Julian Adlard, Munaza Ahmed, Daniel Barrowdale, Rosemarie Davidson, Alan Donaldson, Ros Eeles, D Gareth Evans, Debra Frost, Alex Henderson, Louise Izatt, Kai-Ren Ong, Valérie Bonadona, Isabelle Coupier, Laurence Faivre, Jean-Pierre Fricker, Paul Gesta, Klaartje van Engelen, Agnes Jager, Fred H Menko, Marian J E Mourits, Christian F Singer, Yen Y Tan, Lenka Foretova, Marie Navratilova, Rita K Schmutzler, Carolina Ellberg, Anne-Marie Gerdes, Trinidad Caldes, Jacques Simard, Edith Olah, Anna Jakubowska, Johanna Rantala, Ana Osorio, John L Hopper, Kelly-Anne Phillips, Roger L Milne, Mary Beth Terry, Catherine Noguès, Christoph Engel, Karin Kast, David E Goldgar, Flora E van Leeuwen, Douglas F Easton, Nadine Andrieu, Matti A Rookus, (2021 Jan 25)
Oral contraceptive use and ovarian cancer risk for BRCA1/2 mutation carriers: an international cohort study.
American journal of obstetrics and gynecology : DOI : S0002-9378(21)00038-7

Résumé

Ovarian cancer risk in BRCA1 and BRCA2 mutation carriers has been shown to decrease with longer duration of oral contraceptive use. Although the effects of using oral contraceptives in the general population are well established (approximately 50% risk reduction in ovarian cancer), the estimated risk reduction in mutation carriers is much less precise because of potential bias and small sample sizes. In addition, only a few studies on oral contraceptive use have examined the associations of duration of use, time since last use, starting age, and calendar year of start with risk of ovarian cancer.

Karaki S, Blanc C, Tran T, Galy-Fauroux I, Mougel A, Dransart E, Anson M, Tanchot C, Gibault L, Lepimpec-Barthes F, Darmotte D, Fabre E, Golub* R, Johannes* L, Tartour* E (2021 Jan 24)

CXCR6 deficiency impairs cancer vaccine efficacy and CD8+ resident memory T-cell recruitment in head and neck and lung tumors

J Immunother CancerBMJ Journals : DOI : 10.1136/jitc-2020-001948

Résumé

Background Resident memory T lymphocytes (T_{RM}) are located in tissues and play an important role in immunosurveillance against tumors. The presence of T_{RM} prior to treatment or their induction is associated to the response to anti-Programmed cell death protein 1 (PD-1)/Programmed death-ligand 1 (PD-L1) immunotherapy and the efficacy of cancer vaccines. Previous work by our group and others has shown that the intranasal route of vaccination allows more efficient induction of these cells in head and neck and lung mucosa, resulting in better tumor protection. The mechanisms of in vivo migration of these cells remains largely unknown, apart from the fact that they express the chemokine receptor CXCR6.

Methods We used *CXCR6*-deficient mice and an intranasal tumor vaccination model targeting the Human Papillomavirus (HPV) E7 protein expressed by the TC-1 lung cancer epithelial cell line. The role of CXCR6 and its ligand, CXCL16, was analyzed using multiparametric cytometric techniques and Luminex assays.

Human biopsies obtained from patients with lung cancer were also included in this study.

Results We showed that CXCR6 was preferentially expressed by $CD8^+$ T_{RM} after vaccination in mice and also on intratumoral $CD8^+$ T_{RM} derived from human lung cancer. We also demonstrate that vaccination of Cxcr6-deficient mice induces a defect in the lung recruitment of antigen-specific $CD8^+$ T cells, preferentially in the T_{RM} subsets. In addition, we found that intranasal vaccination with a cancer vaccine is less effective in these *Cxcr6*-deficient mice compared with wild-type mice, and this loss of efficacy is associated with decreased recruitment of local antitumor $CD8^+$ T_{RM}. Interestingly, intranasal, but not intramuscular vaccination induced higher and more sustained concentrations of CXCL16, compared with other chemokines, in the bronchoalveolar lavage fluid and pulmonary parenchyma.

Conclusions This work demonstrates the in vivo role of CXCR6-CXCL16 axis in the migration of $CD8^+$ resident memory T cells in lung mucosa after vaccination, resulting in the control of tumor growth. This work reinforces and explains why the intranasal route of vaccination is the most appropriate strategy for inducing these cells in the head and neck and pulmonary mucosa, which remains a major objective to overcome resistance to anti-PD-1/PD-L1, especially in cold tumors.

Samyuktha Suresh, Solène Huard, Thierry Dubois (2021 Jan 24)

CARM1/PRMT4: Making Its Mark beyond Its Function as a Transcriptional Coactivator.

Trends in cell biology : DOI : 10.1016/j.tcb.2020.12.010

Résumé

Coactivator-associated arginine methyltransferase 1 (CARM1), identified 20 years ago as a coregulator of transcription, is an enzyme that catalyzes arginine methylation of proteins. Beyond its well-established involvement in the regulation of transcription, the physiological functions of CARM1 are still poorly understood. However, recent studies have revealed novel roles of CARM1 in autophagy, metabolism, paraspeckles, and early development. In addition, CARM1 is emerging as an attractive therapeutic target and a drug response biomarker for certain types of cancer. Here, we provide a comprehensive overview of the structure of CARM1 and its post-translational modifications, its various functions, apart from transcriptional coactivation, and its involvement in cancer.

Andrés Ernesto Zucchetti, Noémie Paillon, Olga Markova, Stéphanie Dogniaux, Claire Hivroz, Julien Husson (2021 Jan 20)

Influence of external forces on actin-dependent T cell protrusions during immune synapse formation.

Biology of the cell : 250-263 : DOI : 10.1111/boc.202000133

Résumé

We have previously observed that in response to antigenic activation, T cells produce actin-rich protrusions that generate forces involved in T cell activation. These forces are influenced by the mechanical properties of antigen-presenting cells (APCs). However, how external forces, which can be produced by APCs, influence the dynamic of the actin protrusion remains unknown. In this study, we quantitatively characterised the effects of external forces in the dynamic of the protrusion grown by activated T cells.

Anna Bigas, Ivan Zanoni, Matthew R Hepworth, Stephanie C Eisenbarth, Seth Lucian Masters, Jonathan Kipnis, Carola G Vinuesa, Kim L Good-Jacobson, Stuart G Tangye, Sayuri Yamazaki, Claire Hivroz, Elia Tait Wojno, Ziv Shulman, Marco Colonna (2021 Jan 19)

JEM career launchpad.
The Journal of experimental medicine : DOI : e20202509

Résumé

Tomasz Chelmicki, Emeline Roger, Aurélie Teissandier, Mathilde Dura, Lorraine Bonneville, Sofia Rucli, François Dossin, Camille Fouassier, Sonia Lameiras, Deborah Bourc'his (2021 Jan 14)
mA RNA methylation regulates the fate of endogenous retroviruses.
Nature : 312-316 : DOI : 10.1038/s41586-020-03135-1

Résumé

Endogenous retroviruses (ERVs) are abundant and heterogenous groups of integrated retroviral sequences that affect genome regulation and cell physiology throughout their RNA-centred life cycle. Failure to repress ERVs is associated with cancer, infertility, senescence and neurodegenerative diseases. Here, using an unbiased genome-scale CRISPR knockout screen in mouse embryonic stem cells, we identify mA RNA methylation as a way to restrict ERVs. Methylation of ERV mRNAs is catalysed by the complex of methyltransferase-like METTL3-METTL14 proteins, and we found that depletion of METTL3-METTL14, along with their accessory subunits WTAP and ZC3H13, led to increased mRNA abundance of intracisternal A-particles (IAPs) and related ERVK elements specifically, by targeting their 5′ untranslated region. Using controlled auxin-dependent degradation of the METTL3-METTL14 enzymatic complex, we showed that IAP mRNA and protein abundance is dynamically and inversely correlated with mA catalysis. By monitoring chromatin states and mRNA stability upon METTL3-METTL14 double depletion, we found that mA methylation mainly acts by reducing the half-life of IAP mRNA, and this occurs by the recruitment of the YTHDF family of mA reader proteins. Together, our results indicate that RNA methylation provides a protective effect in maintaining cellular integrity by clearing reactive ERV-derived RNA species, which may be especially important when transcriptional silencing is less stringent.

Andreia Mendes, Julien P Gigan, Christian Rodriguez Rodrigues, Sébastien A Choteau, Doriane Sanseau, Daniela Barros, Catarina Almeida, Voahirana Camosseto, Lionel Chasson, Adrienne W Paton, James C Paton, Rafael J Argüello, Ana-Maria Lennon-Duménil, Evelina Gatti, Philippe Pierre (2021 Jan 14)
Proteostasis in dendritic cells is controlled by the PERK signaling axis independently of ATF4.
Life science alliance : DOI : e202000865

Résumé

In stressed cells, phosphorylation of eukaryotic initiation factor 2α (eIF2α) controls transcriptome-wide changes in mRNA translation and gene expression known as the integrated stress response. We show here that DCs are characterized by high eIF2α

phosphorylation, mostly caused by the activation of the ER kinase PERK (EIF2AK3). Despite high p-eIF2α levels, DCs display active protein synthesis and no signs of a chronic integrated stress response. This biochemical specificity prevents translation arrest and expression of the transcription factor ATF4 during ER-stress induction by the subtilase cytotoxin (SubAB). PERK inactivation, increases globally protein synthesis levels and regulates IFN-β expression, while impairing LPS-stimulated DC migration. Although the loss of PERK activity does not impact DC development, the cross talk existing between actin cytoskeleton dynamics; PERK and eIF2α phosphorylation is likely important to adapt DC homeostasis to the variations imposed by the immune contexts.

Florian Constanty, Alena Shkumatava (2021 Jan 14)

lncRNAs in development and differentiation: from sequence motifs to functional characterization.

Development (Cambridge, England) : DOI : dev182741

Résumé

The number of long noncoding RNAs (lncRNAs) with characterized developmental and cellular functions continues to increase, but our understanding of the molecular mechanisms underlying lncRNA functions, and how they are dictated by RNA sequences, remains limited. Relatively short, conserved sequence motifs embedded in lncRNA transcripts are often important determinants of lncRNA localization, stability and interactions. Identifying such RNA motifs remains challenging due to the substantial length of lncRNA transcripts and the rapid evolutionary turnover of lncRNA sequences. Nevertheless, the recent discovery of specific RNA elements, together with their experimental interrogation, has enabled the first step in classifying heterogeneous lncRNAs into sub-groups with similar molecular mechanisms and functions. In this Review, we focus on lncRNAs with roles in development, cell differentiation and normal physiology in vertebrates, and we discuss the sequence elements defining their functions. We also summarize progress on the discovery of regulatory RNA sequence elements, as well as their molecular functions and interaction partners.

Oksana Reznichenko, Anne Cucchiarini, Valérie Gabelica, Anton Granzhan (2021 Jan 14)

Quadruplex DNA-guided ligand selection from dynamic combinatorial libraries of acylhydrazones

Organic and Biomolecular Chemistry : 19 : 379-386 : DOI : 10.1039/D0OB01908A

Résumé

Dynamic combinatorial libraries of acylhydrazones were prepared from diacylhydrazides and several cationic or neutral aldehydes in the presence of 5-methoxyanthranilic acid catalyst. Pull-down experiments with magnetic beads functionalized with a G-quadruplex (G4)-forming oligonucleotide led to the identification of putative ligands, which were resynthesized or emulated by close structural analogues. G4-binding properties of novel derivatives were

assessed by fluorimetric titrations, mass spectrometry and thermal denaturation experiments, giving evidence of strong binding (K_d < 10 nM) for two compounds.