Year of publication 2016

Catharina von Nicolai, Åsa Ehlén, Charlotte Martin, Xiaodong Zhang, Aura Carreira (2016 Sep 15)

A second DNA binding site in human BRCA2 promotes homologous recombination.

Nature communications : 12813 : DOI : 10.1038/ncomms12813

Summary

BRCA2 tumour-suppressor protein is well known for its role in DNA repair by homologous recombination (HR); assisting the loading of RAD51 recombinase at DNA double-strand breaks. This function is executed by the C-terminal DNA binding domain (CTD) which binds single-stranded (ss)DNA, and the BRC repeats, which bind RAD51 and modulate its assembly onto ssDNA. Paradoxically, analysis of cells resistant to DNA damaging agents missing the CTD restore HR proficiency, suggesting another domain may take over its function. Here, we identify a region in the N terminus of BRCA2 that exhibits DNA binding activity (NTD) and provide evidence for NTD promoting RAD51-mediated HR. A missense variant detected in breast cancer patients located in the NTD impairs HR stimulation on dsDNA/ssDNA junction containing substrates. These findings shed light on the function of the N terminus of BRCA2 and have implications for the evaluation of breast cancer variants.

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Year of publication 2015

Serena Sanulli, Neil Justin, Aurélie Teissandier, Katia Ancelin, Manuela Portoso, Matthieu Caron, Audrey Michaud, Berangère Lombard, Simao T da Rocha, John Offer, Damarys Loew, Nicolas Servant, Michel Wassef, Fabienne Burlina, Steve J Gamblin, Edith Heard, Raphaël Margueron (2015 Mar 5)

Jarid2 Methylation via the PRC2 Complex Regulates H3K27me3 Deposition during Cell Differentiation.

Molecular cell : 769-83 : DOI : 10.1016/j.molcel.2014.12.020

Summary

Polycomb Group (PcG) proteins maintain transcriptional repression throughout development, mostly by regulating chromatin structure. Polycomb Repressive Complex 2 (PRC2), a component of the Polycomb machinery, is responsible for the methylation of histone H3 lysine 27 (H3K27me2/3). Jarid2 was previously identified as a cofactor of PRC2, regulating PRC2 targeting to chromatin and its enzymatic activity. Deletion of Jarid2 leads to impaired orchestration of gene expression during cell lineage commitment. Here, we reveal an unexpected crosstalk between Jarid2 and PRC2, with Jarid2 being methylated by PRC2. This modification is recognized by the Eed core component of PRC2 and triggers an allosteric activation of PRC2's enzymatic activity. We show that Jarid2 methylation is important to

promote PRC2 activity at a locus devoid of H3K27me3 and for the correct deposition of this mark during cell differentiation. Our results uncover a regulation loop where Jarid2 methylation fine-tunes PRC2 activity depending on the chromatin context.

Year of publication 2014

Laura Picas, Julien Viaud, Kristine Schauer, Stefano Vanni, Karim Hnia, Vincent Fraisier, Aurélien Roux, Patricia Bassereau, Frédérique Gaits-Iacovoni, Bernard Payrastre, Jocelyn Laporte, Jean-Baptiste Manneville, Bruno Goud (2014 Dec 9)

BIN1/M-Amphiphysin2 induces clustering of phosphoinositides to recruit its downstream partner dynamin

Nature Communications5 : DOI : 10.1038/ncomms6647

Summary

Phosphoinositides play a central role in many physiological processes by assisting the recruitment of proteins to membranes through specific phosphoinositide-binding motifs. How this recruitment is coordinated in space and time is not well understood. Here we show that BIN1/M-Amphiphysin2, a protein involved in T-tubule biogenesis in muscle cells and frequently mutated in centronuclear myopathies, clusters PtdIns(4,5)P_2 to recruit its downstream partner dynamin. By using several mutants associated with centronuclear myopathies, we find that the N-BAR and the SH3 domains of BIN1 control the kinetics and the accumulation of dynamin on membranes, respectively. We show that phosphoinositide clustering is a mechanism shared by other proteins that interact with PtdIns(4,5)P_2, but do not contain a BAR domain. Our numerical simulations point out that clustering is a diffusion-driven process in which phosphoinositide molecules are not sequestered. We propose that this mechanism plays a key role in the recruitment of downstream phosphoinositide-binding proteins.

Abdeslam Et Taouil, Emilie Brun, Patricia Duchambon, Yves Blouquit, Manon Gilles, Emmanuel Maisonhaute, Cécile Sicard-Roselli (2014 Oct 14)

How protein structure affects redox reactivity: example of Human centrin 2.

Physical chemistry chemical physics : PCCP : 16 : 24493-24498 : DOI : 10.1039/c4cp03536d

Summary

Electron transfer inside proteins plays a central role in their reactivity and biological functions. Herein, we developed a combined approach by gamma radiolysis and electrochemistry which allowed a deep insight into the reactivity of Human centrin 2, a protein very sensitive to oxidative stress and involved in several key biological processes. This protein bears a single terminal tyrosine and was observed to be extremely sensitive to ionizing radiation sources, leading to a tyrosine dimer. By cyclic voltammetry in the 100-1000 V s(-1) range, its redox potential and dimerization rate could be evaluated. Accordingly, reaction in solution with a redox mediator revealed an efficient catalysis. Finally,

protein denaturation by a progressive increase in temperature was proportional to a decrease of dimerization radiolytic yield. Our results thus demonstrated that the protein structure plays a major role in oxidation sensitivity. This leads to meaningful results to understand protein redox reactivity.

Year of publication 2013

Xavier Lahaye, Takeshi Satoh, Matteo Gentili, Silvia Cerboni, Cécile Conrad, Ilse Hurbain, Ahmed El Marjou, Christine Lacabaratz, Jean-Daniel Lelièvre, Nicolas Manel (2013 Sep 6)

The capsids of HIV-1 and HIV-2 determine immune detection of the viral cDNA by the innate sensor cGAS in dendritic cells.

Immunity : 1132-42 : DOI : 10.1016/j.immuni.2013.11.002

Summary

HIV-2 is less pathogenic for humans than HIV-1 and might provide partial cross-protection from HIV-1-induced pathology. Although both viruses replicate in the T cells of infected patients, only HIV-2 replicates efficiently in dendritic cells (DCs) and activates innate immune pathways. How HIV is sensed in DC is unknown. Capsid-mutated HIV-2 revealed that sensing by the host requires viral cDNA synthesis, but not nuclear entry or genome integration. The HIV-1 capsid prevented viral cDNA sensing up to integration, allowing the virus to escape innate recognition. In contrast, DCs sensed capsid-mutated HIV-1 and enhanced stimulation of T cells in the absence of productive infection. Finally, we found that DC sensing of HIV-1 and HIV-2 required the DNA sensor cGAS. Thus, the HIV capsid is a determinant of innate sensing of the viral cDNA by cGAS in dendritic cells. This pathway might potentially be harnessed to develop effective vaccines against HIV-1.

Baligh Miladi, Cyrine Dridi, Ahmed El Marjou, Guilhem Boeuf, Hassib Bouallagui, Florence Dufour, Patrick Di Martino, Abdellatif Elm'selmi (2013 Jun 20)

An improved strategy for easy process monitoring and advanced purification of recombinant proteins.

Molecular biotechnology : 227-35 : DOI : 10.1007/s12033-013-9673-5

Summary

In this work, a multifunctional expression cassette, termed Multitags, combining different and complementary functionalities, was designed and used to monitor the expression and the purification of two model proteins (Pfu DNA polymerase and Myosin-VIIa- and Rab-Interracting protein : MyRIP). Multitags contains two affinity purification tags, a polyhistidine sequence (10× His) and the streptavidin-binding peptide (SBP) and as a marker tag the heme-binding domain of rat cytochrome b5 followed by the TEV cleavage site. Using the Multitags as fusion partner, more than 90 % of both fusion proteins were produced in soluble form when expressed in Escherichia coli KRX. In addition, high purity (99 %) of recombinant proteins was achieved after two consecutive affinity purification steps. The expression

cassette also demonstrated an accurate monitoring capability comparable to that of a dual recognition-based method. The choice of the SBP tag was considered as an integral process that included a method for tag removal. Thus, an immobilized TEV protease fixed on streptavidin-agarose matrix was used for the cleavage of fusion proteins. After digestion, both unprocessed fusion proteins and Multitags were retained on the proteolytic column via their SBP sequence, allowing cleavage and recovery of target proteins on one step. This combined approach may accelerate the development of optimized production processes, while insuring high product quality and a low production cost.

Monique Chan-Huot, Philippe Lesot, Philippe Pelupessy, Luminita Duma, Geoffrey Bodenhausen, Patricia Duchambon, Michael D Toney, U Venkateswara Reddy, N Suryaprakash (2013 Apr 23)
“On-the-fly” kinetics of enzymatic racemization using deuterium NMR in DNA-based chiral oriented media.
Analytical chemistry : 4694-7 : DOI : 10.1021/ac4004002

Summary

We report the in situ and real-time monitoring of the interconversion of L- and D-alanine-d3 by alanine racemase from Bacillus stearothermophilus directly observed by (2)H NMR spectroscopy in anisotropic phase. The enantiomers are distinguished by the difference of their (2)H quadrupolar splittings in a chiral liquid crystal containing short DNA fragments. The proof-of-principle, the reliability, and the robustness of this new method is demonstrated by the determination of the turnover rates of the enzyme using the Michaelis-Menten model.