Année de publication : 2019

Emilia Puig Lombardi, Allyson Holmes, Daniela Verga, Marie-Paule Teulade-Fichou, Alain Nicolas, Arturo Londoño-Vallejo (2019 Jul 9)

Thermodynamically stable and genetically unstable G-quadruplexes are depleted in genomes across species.

Nucleic acids research : 47 : 6098-6113 : DOI : 10.1093/nar/gkz463

Résumé

G-quadruplexes play various roles in multiple biological processes, which can be positive when a G4 is involved in the regulation of gene expression or detrimental when the folding of a stable G4 impairs DNA replication promoting genome instability. This duality interrogates the significance of their presence within genomes. To address the potential biased evolution of G4 motifs, we analyzed their occurrence, features and polymorphisms in a large spectrum of species. We found extreme bias of the short-looped G4 motifs, which are the most thermodynamically stable in vitro and thus carry the highest folding potential in vivo. In the human genome, there is an over-representation of single-nucleotide-loop G4 motifs (G4-L1), which are highly conserved among humans and show a striking excess of the thermodynamically least stable G4-L1A (G3AG3AG3AG3) sequences. Functional assays in yeast showed that G4-L1A caused the lowest levels of both spontaneous and G4-ligand-induced instability. Analyses across 600 species revealed the depletion of the most stable G4-L1C/T quadruplexes in most genomes in favor of G4-L1A in vertebrates or G4-L1G in other eukaryotes. We discuss how these trends might be the result of species-specific mutagenic processes associated to a negative selection against the most stable motifs, thus neutralizing their detrimental effects on genome stability while preserving positive G4-associated biological roles.

Emilia Puig Lombardi, Arturo Londoño-Vallejo, Alain Nicolas (2019 May 30)

Relationship Between G-Quadruplex Sequence Composition in Viruses and Their Hosts.

Molecules (Basel, Switzerland) : DOI : E1942

Résumé

A subset of guanine-rich nucleic acid sequences has the potential to fold into G-quadruplex (G4) secondary structures, which are functionally important for several biological processes, including genome stability and regulation of gene expression. Putative quadruplex sequences (PQSs) GNGNGNG are widely found in eukaryotic and prokaryotic genomes, but the base composition of the N loops is biased across species. Since the viruses partially hijack their hosts' cellular machinery for proliferation, we examined the PQS motif size, loop length, and nucleotide compositions of 7370 viral genome assemblies and compared viral and host PQS motifs. We studied seven viral taxa infecting five distant eukaryotic hosts and created a resource providing a comprehensive view of the viral quadruplex motifs. Overall, short-looped PQSs are predominant and with a similar composition across viral taxonomic

groups, albeit subtle trends emerge upon classification by hosts. Specifically, there is a higher frequency of pyrimidine loops in viruses infecting animals irrespective of the viruses' genome type. This observation is confirmed by an in-depth analysis of the Herpesviridae family of viruses, which showed a distinctive accumulation of thermally stable C-looped quadruplexes in viruses infecting high-order vertebrates. The occurrence of viral C-looped G4s, which carry binding sites for host transcription factors, as well as the high prevalence of viral TTA-looped G4s, which are identical to vertebrate telomeric motifs, provide concrete examples of how PQSs may help viruses impinge upon, and benefit from, host functions. More generally, these observations suggest a co-evolution of virus and host PQSs, thus underscoring the potential functional significance of G4s.

Pinskaya M., Saci Z., Gallopin M., Nguyen N.H., Gabriel M., Firlej V., Descrimes M., de la Taille A., Londo~no-Vallejo A., Allory Y., Gautheret D., Morillon A. (2019 Jan 1)

Blind exploration of the unreferenced transcriptome reveals novel RNAs for prostate cancer diagnosis

bioRxiv : DOI : 10.1101/644104

Résumé

The broad use of RNA-sequencing technologies held a promise of improved diagnostic tools based on comprehensive transcript sets. However, mining human transcriptome data for disease biomarkers in clinical specimens is restricted by the limited power of conventional reference-based protocols relying on uniquely mapped reads and transcript annotations. Here, we implemented a blind reference-free computational protocol, DE-kupl, to directly infer RNA variations of any origin, including yet unreferenced RNAs, from high coverage total stranded RNA-sequencing datasets of tissue origin. As a bench test, this protocol was powered for detection of RNA subsequences embedded into unannotated putative long noncoding (lnc)RNAs expressed in prostate cancer tissues. Through filtering and visual inspection of 1,179 candidates, we defined 21 lncRNA probes that were further validated for robust tumor-specific expression by NanoString single molecule-based RNA measurements in 144 tissue specimens. Predictive modeling yielded a restricted probe panel enabling over 90% of true positive detection of cancer in an independent dataset from The Cancer Genome Atlas. Remarkably, this clinical signature made of only 9 unannotated lncRNAs largely outperformed PCA3, the only RNA biomarker approved by the Food and Drug Administration agency, specifically, in detection of high-risk prostate tumors. The proposed reference-free computational workflow is modular, highly sensitive and robust and can be applied to any pathology and any clinical application.

Année de publication : 2018

Porreca RM, Glousker G, Awad A, Matilla Fernandez MI, Gibaud A, Naucke C, Cohen SB, Bryan TM, Tzfati Y, Draskovic I, Londoño-Vallejo A (2018 Mar 7)

Human RTEL1 stabilizes long G-overhangs allowing telomerase-dependent over-

extension
Nucleic Acids Research : DOI : 10.1093/nar/gky173

Résumé

Telomere maintenance protects the cell against genome instability and senescence. Accelerated telomere attrition is a characteristic of premature aging syndromes including Dyskeratosis congenita (DC). Mutations in hRTEL1 are associated with a severe form of DC called Hoyeraal-Hreidarsson syndrome (HHS). HHS patients carry short telomeres and HHS cells display telomere damage. Here we investigated how hRTEL1 contributes to telomere maintenance in human primary as well as tumor cells. Transient depletion of hRTEL1 resulted in rapid telomere shortening only in the context of telomerase-positive cells with very long telomeres and high levels of telomerase. The effect of hRTEL1 on telomere length is telomerase dependent without impacting telomerase biogenesis or targeting of the enzyme to telomeres. Instead, RTEL1 depletion led to a decrease in both G-overhang content and POT1 association with telomeres with limited telomere uncapping. Strikingly, overexpression of POT1 restored telomere length but not the overhang, demonstrating that G-overhang loss is the primary defect caused by RTEL1 depletion. We propose that hRTEL1 contributes to the maintenance of long telomeres by preserving long G-overhangs, thereby facilitating POT1 binding and elongation by telomerase.

Année de publication : 2017

Jahn A, Rane G, Paszkowski-Rogacz M, Sayols S, Bluhm A, Han CT, Draškovič I, Londoño-Vallejo JA, Kumar AP, Buchholz F, Butter F, Kappei D (2017 Jun 1)
ZBTB48 is both a vertebrate telomere-binding protein and a transcriptional activator
EMBO Report : 18(6) : DOI : 10.15252/embr.201744095

Résumé

Telomeres constitute the ends of linear chromosomes and together with the shelterin complex form a structure essential for genome maintenance and stability. In addition to the constitutive binding of the shelterin complex, other direct, yet more transient interactions are mediated by the CST complex and HOT1/HMBOX1, while subtelomeric variant repeats are recognized by NR2C/F transcription factors. Recently, the Kruppel-like zinc finger protein ZBTB48/HKR3/TZAP has been described as a novel telomere-associated factor in the vertebrate lineage. Here, we show that ZBTB48 binds directly both to telomeric and to subtelomeric variant repeat sequences. ZBTB48 is found at telomeres of human cancer cells regardless of the mode of telomere maintenance and it acts as a negative regulator of telomere length. In addition to its telomeric function, we demonstrate through a combination of RNAseq, ChIPseq and expression proteomics experiments that ZBTB48 acts as a transcriptional activator on a small set of target genes, including mitochondrial fission process 1 (MTFP1). This discovery places ZBTB48 at the interface of telomere length regulation, transcriptional control and mitochondrial metabolism.

Bruno Teste, Jerome Champ, Arturo Londono-Vallejo, Stéphanie Descroix, Laurent Malaquin, Jean-Louis Viovy, Irena Draskovic, Guillaume Mottet (2017 Jan 17)

Chromatin immunoprecipitation in microfluidic droplets: towards fast and cheap analyses.

Lab on a chip : 530-537 : DOI : 10.1039/c6lc01535b

Résumé

Genetic organization is governed by the interaction of DNA with histone proteins, and differential modifications of these proteins is a fundamental mechanism of gene regulation. Histone modifications are primarily studied through chromatin immunoprecipitation (ChIP) assays, however conventional ChIP procedures are time consuming, laborious and require a large number of cells. Here we report for the first time the development of ChIP in droplets based on a microfluidic platform combining nanoliter droplets, magnetic beads (MB) and magnetic tweezers (MT). The droplet approach enabled compartmentalization and improved mixing, while reducing the consumption of samples and reagents in an integrated workflow. Anti-histone antibodies grafted to MB were used as a solid support to capture and transfer the target chromatin from droplets to droplets in order to perform chromatin immunoprecipitation, washing, elution and purification of DNA. We designed a new ChIP protocol to investigate four different types of modified histones with known roles in gene activation or repression. We evaluated the performances of this new ChIP in droplet assay in comparison with conventional methods. The proposed technology dramatically reduces analytical time from a few days to 7 hours, simplifies the ChIP protocol and decreases the number of cells required by 100 fold while maintaining a high degree of sensitivity and specificity. Therefore this droplet-based ChIP assay represents a new, highly advantageous and convenient approach to epigenetic analyses.

Ourliac-Garnier I, Londoño-Vallejo A (2017 Jan 1)

Telomere Length Analysis by Quantitative Fluorescent in Situ Hybridization (Q-FISH)

Methods in Molecular Biology : 29-39 : DOI : 10.1007/978-1-4939-6892-3_3

Résumé

Length is a functional parameter of telomeres, the nucleoprotein structures that protect chromosome ends. The availability of highly specific, high affinity probes for telomeric repeat sequences allowed the development of quantitative approaches aimed at measuring telomere length directly on chromosomes or in interphase nuclei. Here, we describe a general method for telomere quantitative FISH on metaphase chromosomes and discuss its most common applications in research

Année de publication : 2016

Robert Jackson, Bruce A Rosa, Sonia Lameiras, Sean Cuninghame, Josee Bernard, Wely B

Floriano, Paul F Lambert, Alain Nicolas, Ingeborg Zehbe (2016 Nov 4)
Functional variants of human papillomavirus type 16 demonstrate host genome integration and transcriptional alterations corresponding to their unique cancer epidemiology.
BMC genomics : 851

Résumé

Human papillomaviruses (HPVs) are a worldwide burden as they are a widespread group of tumour viruses in humans. Having a tropism for mucosal tissues, high-risk HPVs are detected in nearly all cervical cancers. HPV16 is the most common high-risk type but not all women infected with high-risk HPV develop a malignant tumour. Likely relevant, HPV genomes are polymorphic and some HPV16 single nucleotide polymorphisms (SNPs) are under evolutionary constraint instigating variable oncogenicity and immunogenicity in the infected host.

Pierre Thouvenot, Lou Fourrière, Elodie Dardillac, Barbara Ben Yamin, Aurianne Lescure, Vincent Lejour, Xavier Heiligenstein, Jean-Baptiste Boulé, Maryse Romao, Graça Raposo-Benedetti, Bernard S Lopez, Alain Nicolas, Gaël A Millot (2016 Nov 2)
Yeast cells reveal the misfolding and the cellular mislocalisation of the human BRCA1 protein.
Journal of cell science : DOI : jcs.192880

Résumé

Understanding the effect of an ever-growing number of human variants detected by genome sequencing is a medical challenge. The yeast Saccharomyces cerevisiae model has held attention for its capacity to monitor the functional impact of missense mutations found in human genes, including the BRCA1 breast/ovarian cancer susceptibility gene. When expressed in yeast, the wild-type full-length BRCA1 protein forms a single nuclear aggregate and induces a growth inhibition. Both events are modified by pathogenic mutations of BRCA1. However, the biological interpretation of these events remains to be determined. Here, we show that the BRCA1 nuclear aggregation and the growth inhibition are sensitive to misfolding effects induced by missense mutations. Moreover, misfolding mutations impair the nuclear targeting of BRCA1 in yeast cells and in a human cell line. In conclusion, we establish a connection between misfolding and nuclear transport impairment and we illustrate that yeast is a suitable model to decipher the effect of misfolding mutations.

Loic Verlingue, Aurélien Dugourd, Gautier Stoll, Emmanuel Barillot, Laurence Calzone, Arturo Londoño-Vallejo (2016 Sep 11)
A comprehensive approach to the molecular determinants of lifespan using a Boolean model of geroconversion.
Aging cell : DOI : 10.1111/acel.12504

Résumé

Altered molecular responses to insulin and growth factors (GF) are responsible for late-life shortening diseases such as type-2 diabetes mellitus (T2DM) and cancers. We have built a network of the signaling pathways that control S-phase entry and a specific type of senescence called geroconversion. We have translated this network into a Boolean model to study possible cell phenotype outcomes under diverse molecular signaling conditions. In the context of insulin resistance, the model was able to reproduce the variations of the senescence level observed in tissues related to T2DM's main morbidity and mortality. Furthermore, by calibrating the pharmacodynamics of mTOR inhibitors, we have been able to reproduce the dose-dependent effect of rapamycin on liver degeneration and lifespan expansion in wild-type and HER2-neu mice. Using the model, we have finally performed an in silico prospective screen of the risk-benefit ratio of rapamycin dosage for healthy lifespan expansion strategies. We present here a comprehensive prognostic and predictive systems biology tool for human aging.

Holmes A., Lameiras S., Jeannot E., Marie Y., Castera L., Sastre-Garau X., Nicolas A. (2016 May 16)

Mechanistic signatures of HPV insertions in cervical carcinomas

Nature Genomic Medicine : DOI : 10.1038/npjgenmed.2016.4

Résumé

Laurent Jullien, Caroline Kannengiesser, Laetitia Kermasson, Valérie Cormier-Daire, Thierry Leblanc, Jean Soulier, Arturo Londono-Vallejo, Jean-Pierre de Villartay, Isabelle Callebaut, Patrick Revy (2016 Feb 6)

Mutations of the RTEL1 Helicase in a Hoyeraal-Hreidarsson Syndrome Patient Highlight the Importance of the ARCH Domain.

Human mutation : 469-72 : DOI : 10.1002/humu.22966

Résumé

The DNA helicase RTEL1 participates in telomere maintenance and genome stability. Biallelic mutations in the RTEL1 gene account for the severe telomere biology disorder characteristic of the Hoyeraal-Hreidarsson syndrome (HH). Here, we report a HH patient (P4) carrying two novel compound heterozygous mutations in RTEL1: a premature stop codon (c.949A>T, p.Lys317*) and an intronic deletion leading to an exon skipping and an in-frame deletion of 25 amino-acids (p.Ile398_Lys422). P4's cells exhibit short and dysfunctional telomeres similarly to other RTEL1-deficient patients. 3D structure predictions indicated that the p.Ile398_Lys422 deletion affects a part of the helicase ARCH domain, which lines the pore formed with the core HD and the iron-sulfur cluster domains and is highly specific of sequences from the eukaryotic XPD family members.

Hervé Técher, Stéphane Koundrioukoff, Sandra Carignon, Therese Wilhelm, Gaël A Millot, Bernard S Lopez, Olivier Brison, Michelle Debatisse (2016 Jan 26)

Signaling from Mus81-Eme2-Dependent DNA Damage Elicited by Chk1 Deficiency Modulates Replication Fork Speed and Origin Usage.

Cell reports : 1114-27 : DOI : 10.1016/j.celrep.2015.12.093

Résumé

Mammalian cells deficient in ATR or Chk1 display moderate replication fork slowing and increased initiation density, but the underlying mechanisms have remained unclear. We show that exogenous deoxyribonucleosides suppress both replication phenotypes in Chk1-deficient, but not ATR-deficient, cells. Thus, in the absence of exogenous stress, depletion of either protein impacts the replication dynamics through different mechanisms. In addition, Chk1 deficiency, but not ATR deficiency, triggers nuclease-dependent DNA damage. Avoiding damage formation through invalidation of Mus81-Eme2 and Mre11, or preventing damage signaling by turning off the ATM pathway, suppresses the replication phenotypes of Chk1-deficient cells. Damage and resulting DDR activation are therefore the cause, not the consequence, of replication dynamics modulation in these cells. Together, we identify moderate reduction of precursors available for replication as an additional outcome of DDR activation. We propose that resulting fork slowing, and subsequent firing of backup origins, helps replication to proceed along damaged templates.

Delphine Benarroch-Popivker, Sabrina Pisano, Aaron Mendez-Bermudez, Liudmyla Lototska, Parminder Kaur, Serge Bauwens, Nadir Djerbi, Chrysa M Latrick, Vincent Fraisier, Bei Pei, Alexandre Gay, Emilie Jaune, Kevin Foucher, Julien Cherfils-Vicini, Eric Aeby, Simona Miron, Arturo Londoño-Vallejo, Jing Ye, Marie-Hélène Le Du, Hong Wang, Eric Gilson, Marie-Josèphe Giraud-Panis (2016 Jan 18)

TRF2-Mediated Control of Telomere DNA Topology as a Mechanism for Chromosome-End Protection.

Molecular cell : 274-86 : DOI : 10.1016/j.molcel.2015.12.009

Résumé

The shelterin proteins protect telomeres against activation of the DNA damage checkpoints and recombinational repair. We show here that a dimer of the shelterin subunit TRF2 wraps ~ 90 bp of DNA through several lysine and arginine residues localized around its homodimerization domain. The expression of a wrapping-deficient TRF2 mutant, named Top-less, alters telomeric DNA topology, decreases the number of terminal loops (t-loops), and triggers the ATM checkpoint, while still protecting telomeres against non-homologous end joining (NHEJ). In Top-less cells, the protection against NHEJ is alleviated if the expression of the TRF2-interacting protein RAP1 is reduced. We conclude that a distinctive topological state of telomeric DNA, controlled by the TRF2-dependent DNA wrapping and linked to t-loop formation, inhibits both ATM activation and NHEJ. The presence of RAP1 at telomeres appears as a backup mechanism to prevent NHEJ when topology-mediated telomere

protection is impaired.

Année de publication : 2015

Mohamed Izikki, Eric Hoang, Irena Draskovic, Olaf Mercier, Florence Lecerf, Lilia Lamrani, Win-Yan Liu, Christophe Guignabert, Elie Fadel, Peter Dorfmuller, Marc Humbert, Arturo Londoño-Vallejo, Saadia Eddahibi (2015 Oct 24)

Telomere Maintenance Is a Critical Determinant in the Physiopathology of Pulmonary Hypertension.

Journal of the American College of Cardiology : 1942-3 : DOI : 10.1016/j.jacc.2015.08.869

Résumé