

Année de publication : 2018

Antoine Hocher, Myriam Ruault, Petra Kaferle, Marc Describes, Mickaël Garnier, Antonin Morillon, Angela Taddei (2018 Oct 26)

Expanding heterochromatin reveals discrete subtelomeric domains delimited by chromatin landscape transitions.

Genome research : [DOI : gr.236554.118](https://doi.org/10.1101/236554)

Résumé

The eukaryotic genome is divided into chromosomal domains of heterochromatin and euchromatin. Transcriptionally silent heterochromatin is found at subtelomeric regions, leading to the telomeric position effect (TPE) in yeast fly and human. Heterochromatin generally initiates and spreads from defined loci, and diverse mechanisms prevent the ectopic spread of heterochromatin into euchromatin. Here, we overexpressed the silencing factor Sir3 at varying levels in yeast and found that Sir3 spreads into Extended Silent Domains (ESDs), eventually reaching saturation at subtelomeres. We observed the spread of Sir3 into subtelomeric domains associated with specific histone marks in wild-type cells and stopping at zones of histone mark transitions including H3K79 tri-methylation levels. Our study shows that the conserved H3K79 methyltransferase Dot1 is essential in restricting Sir3 spread beyond ESDs, thus ensuring viability upon overexpression of Sir3. Lastly, our analyses of published data demonstrate how ESDs unveil uncharacterized discrete domains isolating structural and functional subtelomeric features from the rest of the genome. Our work offers a new approach on how to separate subtelomeres from the core chromosome.

Année de publication : 2017

Amandine Batté, Clémentine Brocas, Hélène Bordelet, Antoine Hocher, Myriam Ruault, Adouda Adjiri, Angela Taddei, Karine Dubrana (2017 Jul 30)

Recombination at subtelomeres is regulated by physical distance, double-strand break resection and chromatin status.

The EMBO journal : 2609-2625 : [DOI : 10.15252/embj.201796631](https://doi.org/10.15252/embj.201796631)

Résumé

Homologous recombination (HR) is a conserved mechanism that repairs broken chromosomes via intact homologous sequences. How different genomic, chromatin and subnuclear contexts influence HR efficiency and outcome is poorly understood. We developed an assay to assess HR outcome by gene conversion (GC) and break-induced replication (BIR), and discovered that subtelomeric double-stranded breaks (DSBs) are preferentially repaired by BIR despite the presence of flanking homologous sequences. Overexpression of a silencing-deficient SIR3 mutant led to active grouping of telomeres and specifically increased the GC efficiency between subtelomeres. Thus, physical distance limits GC at subtelomeres. However, the repair efficiency between reciprocal intrachromosomal and subtelomeric sequences varies up to 15-fold, depending on the location of the DSB,

indicating that spatial proximity is not the only limiting factor for HR EXO1 deletion limited the resection at subtelomeric DSBs and improved GC efficiency. The presence of repressive chromatin at subtelomeric DSBs also favoured recombination, by counteracting EXO1-mediated resection. Thus, repressive chromatin promotes HR at subtelomeric DSBs by limiting DSB resection and protecting against genetic information loss.

Eldad Kepten, Judith Miné-Hattab (2017 Feb 28)

[Lamin A: a key protein in chromatin motion].

Medecine sciences : M/S : 126-130 : [DOI : 10.1051/medsci/20173302004](https://doi.org/10.1051/medsci/20173302004)

Résumé

Année de publication : 2015

Ivaylo Nikolov, Angela Taddei (2015 Oct 30)

Linking replication stress with heterochromatin formation.

Chromosoma : 523-33 : [DOI : 10.1007/s00412-015-0545-6](https://doi.org/10.1007/s00412-015-0545-6)

Résumé

The eukaryotic genome can be roughly divided into euchromatin and heterochromatin domains that are structurally and functionally distinct. Heterochromatin is characterized by its high compaction that impedes DNA transactions such as gene transcription, replication, or recombination. Beyond its role in regulating DNA accessibility, heterochromatin plays essential roles in nuclear architecture, chromosome segregation, and genome stability. The formation of heterochromatin involves special histone modifications and the recruitment and spreading of silencing complexes that impact the higher-order structures of chromatin; however, its molecular nature varies between different chromosomal regions and between species. Although heterochromatin has been extensively characterized, its formation and maintenance throughout the cell cycle are not yet fully understood. The biggest challenge for the faithful transmission of chromatin domains is the destabilization of chromatin structures followed by their reassembly on a novel DNA template during genomic replication. This destabilizing event also provides a window of opportunity for the de novo establishment of heterochromatin. In recent years, it has become clear that different types of obstacles such as tight protein-DNA complexes, highly transcribed genes, and secondary DNA structures could impede the normal progression of the replisome and thus have the potential to endanger the integrity of the genome. Multiple studies carried out in different model organisms have demonstrated the capacity of such replisome impediments to favor the formation of heterochromatin. Our review summarizes these reports and discusses the potential role of replication stress in the formation and maintenance of heterochromatin and the role that silencing proteins could play at sites where the integrity of the genome is compromised.

Compartmentation et dynamique des fonctions nucléaires

Micol Guidi, Myriam Ruault, Martial Marbouty, Isabelle Loïodice, Axel Cournac, Cyrille Billaudeau, Antoine Hocher, Julien Mozziconacci, Romain Koszul, Angela Taddei (2015 Apr 2)

Spatial reorganization of telomeres in long-lived quiescent cells.

Genome biology : 206 : [DOI : 10.1186/s13059-015-0766-2](https://doi.org/10.1186/s13059-015-0766-2)

Résumé

The spatiotemporal behavior of chromatin is an important control mechanism of genomic function. Studies in *Saccharomyces cerevisiae* have broadly contributed to demonstrate the functional importance of nuclear organization. Although in the wild yeast survival depends on their ability to withstand adverse conditions, most of these studies were conducted on cells undergoing exponential growth. In these conditions, as in most eukaryotic cells, silent chromatin that is mainly found at the 32 telomeres accumulates at the nuclear envelope, forming three to five foci.

Année de publication : 2014

Isabelle Loïodice, Marion Dubarry, Angela Taddei (2014 Mar 11)

Scoring and manipulating gene position and dynamics using FROS in budding yeast.

Current protocols in cell biology / editorial board, Juan S. Bonifacino ... [et al.] : Unit 22.17.1-14 : [DOI : 10.1002/0471143030.cb2217s62](https://doi.org/10.1002/0471143030.cb2217s62)

Résumé

The spatial organization of the genome within the nucleus is now seen as a key contributor to genome function. Studying chromatin dynamics in living cells has been rendered possible by the development of fast microscopy coupled with fluorescent repressor operator systems (FROS). In these systems, arrays of protein-binding sites integrated at specific loci by homologous recombination are monitored through the fluorescence of tagged DNA-binding proteins. In the budding yeast, where homologous recombination is efficient, this technique, combined with targeting assay and genetic analysis, has been extremely powerful for studying the determinants and function of chromatin dynamics in living cells. However, issues have been recurrently raised in different species regarding the use of these systems. Here we discuss the different uses of gene tagging with FROS and their limitations, focusing in budding yeast as a model organism.

Année de publication : 2013

Nathanaël Hozé, Myriam Ruault, Carlo Amoruso, Angela Taddei, David Holcman (2013 Apr 10)

Spatial telomere organization and clustering in yeast *Saccharomyces cerevisiae* nucleus is generated by a random dynamics of aggregation-dissociation.

Molecular biology of the cell : 1791-800, S1-10 : [DOI : 10.1091/mbc.E13-01-0031](https://doi.org/10.1091/mbc.E13-01-0031)

Résumé

Spatial and temporal behavior of chromosomes and their regulatory proteins is a key control mechanism in genomic function. This is exemplified by the clustering of the 32 budding yeast telomeres that form foci in which silencing factors concentrate. To uncover the determinants of telomere distribution, we compare live-cell imaging with a stochastic model of telomere dynamics that we developed. We show that random encounters alone are inadequate to produce the clustering observed in vivo. In contrast, telomere dynamics observed in vivo in both haploid and diploid cells follows a process of dissociation-aggregation. We determine the time that two telomeres spend in the same cluster for the telomere distribution observed in cells expressing different levels of the silencing factor Sir3 protein, limiting for telomere clustering. We conclude that telomere clusters, their dynamics, and their nuclear distribution result from random motion, aggregation, and dissociation of telomeric regions, specifically determined by the amount of Sir3.

Peter Meister, Angela Taddei (2013 Jan 15)

Building silent compartments at the nuclear periphery: a recurrent theme.

Current opinion in genetics & development : 96-103 : [DOI : 10.1016/j.gde.2012.12.001](https://doi.org/10.1016/j.gde.2012.12.001)

Résumé

In eukaryotes, the genetic material is stored in the nucleus, which is enclosed in a double lipid bilayer, the nuclear envelope (NE). It protects the genome from physical stress and separates it from the rest of the cell. On top of this physical function, growing evidence shows that the nuclear periphery contributes to the 3D organization of the genome. In turn, tridimensional organization of chromatin in the nuclear space influences genome expression. Here we review recent findings on the function of this physical barrier in gene repression and latest models on how silent subnuclear compartments at the NE are built in yeast as well as in the nematode *C. elegans* and mammalian cells; trying to draw parallels between the three systems.

Année de publication : 2012

Angela Taddei, Susan M Gasser (2012 Sep 12)

Structure and function in the budding yeast nucleus.

Genetics : 107-29 : [DOI : 10.1534/genetics.112.140608](https://doi.org/10.1534/genetics.112.140608)

Résumé

Budding yeast, like other eukaryotes, carries its genetic information on chromosomes that are sequestered from other cellular constituents by a double membrane, which forms the nucleus. An elaborate molecular machinery forms large pores that span the double membrane and regulate the traffic of macromolecules into and out of the nucleus. In multicellular eukaryotes, an intermediate filament meshwork formed of lamin proteins

Compartmentation et dynamique des fonctions nucléaires

bridges from pore to pore and helps the nucleus reform after mitosis. Yeast, however, lacks lamins, and the nuclear envelope is not disrupted during yeast mitosis. The mitotic spindle nucleates from the nucleoplasmic face of the spindle pole body, which is embedded in the nuclear envelope. Surprisingly, the kinetochores remain attached to short microtubules throughout interphase, influencing the position of centromeres in the interphase nucleus, and telomeres are found clustered in foci at the nuclear periphery. In addition to this chromosomal organization, the yeast nucleus is functionally compartmentalized to allow efficient gene expression, repression, RNA processing, genomic replication, and repair. The formation of functional subcompartments is achieved in the nucleus without intranuclear membranes and depends instead on sequence elements, protein-protein interactions, specific anchorage sites at the nuclear envelope or at pores, and long-range contacts between specific chromosomal loci, such as telomeres. Here we review the spatial organization of the budding yeast nucleus, the proteins involved in forming nuclear subcompartments, and evidence suggesting that the spatial organization of the nucleus is important for nuclear function.

Frank R Neumann, Vincent Dion, Lutz R Gehlen, Monika Tsai-Pflugfelder, Roger Schmid, Angela Taddei, Susan M Gasser (2012 Feb 21)

Targeted INO80 enhances subnuclear chromatin movement and ectopic homologous recombination.

Genes & development : 369-83 : [DOI : 10.1101/gad.176156.111](https://doi.org/10.1101/gad.176156.111)

Résumé

Chromatin in the interphase nucleus moves in a constrained random walk. Despite extensive study, the molecular causes of such movement and its impact on DNA-based reactions are unclear. Using high-precision live fluorescence microscopy in budding yeast, we quantified the movement of tagged chromosomal loci to which transcriptional activators or nucleosome remodeling complexes were targeted. We found that local binding of the transcriptional activator VP16, but not of the Gal4 acidic domain, enhances chromatin mobility. The increase in movement did not correlate strictly with RNA polymerase II (PolII) elongation, but could be phenocopied by targeting the INO80 remodeler to the locus. Enhanced chromatin mobility required Ino80's ATPase activity. Consistently, the INO80-dependent remodeling of nucleosomes upon transcriptional activation of the endogenous PHO5 promoter enhanced chromatin movement locally. Finally, increased mobility at a double-strand break was also shown to depend in part on the INO80 complex. This correlated with increased rates of spontaneous gene conversion. We propose that local chromatin remodeling and nucleosome eviction increase large-scale chromatin movements by enhancing the flexibility of the chromatin fiber.

Année de publication : 2011

Marion Dubarry, Isabelle Loïodice, Chunlong L Chen, Claude Thermes, Angela Taddei (2011 Jul 5)
Tight protein-DNA interactions favor gene silencing.

Genes & development : 1365-70 : [DOI : 10.1101/gad.611011](https://doi.org/10.1101/gad.611011)

Résumé

The heterochromatin-like structure formed by the yeast silent information regulator complex (SIR) represses transcription at the silent mating type loci and telomeres. Here, we report that tight protein-DNA complexes induce ectopic recruitment of the SIR complex, promoting gene silencing and changes in subnuclear localization when cis-acting elements are nearby. Importantly, lack of the replication fork-associated helicase Rrm3 enhances this induced gene repression. Additionally, Sir3 and Sir4 are enriched genome-wide at natural replication pause sites, including tRNA genes. Consistently, inserting a tRNA gene promotes SIR-mediated silencing of a nearby gene. These results reveal that replication stress arising from tight DNA-protein interactions favors heterochromatin formation.

Myriam Ruault, Arnaud De Meyer, Isabelle Loïodice, Angela Taddei (2011 Feb 9)

Clustering heterochromatin: Sir3 promotes telomere clustering independently of silencing in yeast.

The Journal of cell biology : 417-31 : [DOI : 10.1083/jcb.201008007](https://doi.org/10.1083/jcb.201008007)

Résumé

A general feature of the nucleus is the organization of repetitive deoxyribonucleic acid sequences in clusters concentrating silencing factors. In budding yeast, we investigated how telomeres cluster in perinuclear foci associated with the silencing complex Sir2-Sir3-Sir4 and found that Sir3 is limiting for telomere clustering. Sir3 overexpression triggers the grouping of telomeric foci into larger foci that relocalize to the nuclear interior and correlate with more stable silencing in subtelomeric regions. Furthermore, we show that Sir3's ability to mediate telomere clustering can be separated from its role in silencing. Indeed, nonacetylatable Sir3, which is unable to spread into subtelomeric regions, can mediate telomere clustering independently of Sir2-Sir4 as long as it is targeted to telomeres by the Rap1 protein. Thus, arrays of Sir3 binding sites at telomeres appeared as the sole requirement to promote trans-interactions between telomeres. We propose that similar mechanisms involving proteins able to oligomerize account for long-range interactions that impact genomic functions in many organisms.

Lutz R Gehlen, Shigeki Nagai, Kenji Shimada, Peter Meister, Angela Taddei, Susan M Gasser (2011 Jan 4)

Nuclear geometry and rapid mitosis ensure asymmetric episome segregation in yeast.

Current biology : CB : 25-33 : [DOI : 10.1016/j.cub.2010.12.016](https://doi.org/10.1016/j.cub.2010.12.016)

Résumé

Asymmetric cell division drives the generation of differentiated cells and maintenance of

stem cells. In budding yeast, autonomously replicating sequence (ARS) plasmids lacking centromere elements are asymmetrically segregated into the mother cell, where they are thought to contribute to cellular senescence. This phenomenon has been proposed to result from the active retention of plasmids through an interaction with nuclear pores.

Année de publication : 2010

Angela Taddei, Heiko Schober, Susan M Gasser (2010 Jun 18)

The budding yeast nucleus.

Cold Spring Harbor perspectives in biology : a000612 : [DOI : 10.1101/cshperspect.a000612](https://doi.org/10.1101/cshperspect.a000612)

Résumé

The budding yeast nucleus, like those of other eukaryotic species, is highly organized with respect to both chromosomal sequences and enzymatic activities. At the nuclear periphery interactions of nuclear pores with chromatin, mRNA, and transport factors promote efficient gene expression, whereas centromeres, telomeres, and silent chromatin are clustered and anchored away from pores. Internal nuclear organization appears to be function-dependent, reflecting localized sites for tRNA transcription, rDNA transcription, ribosome assembly, and DNA repair. Recent advances have identified new proteins involved in the positioning of chromatin and have allowed testing of the functional role of higher-order chromatin organization. The unequal distribution of silent information regulatory factors and histone modifying enzymes, which arises in part from the juxtaposition of telomeric repeats, has been shown to influence chromatin-mediated transcriptional repression. Other localization events suppress unwanted recombination. These findings highlight the contribution budding yeast genetics and cytology have made to dissecting the functional role of nuclear structure.

Takahito Yoshida, Kenji Shimada, Yukako Oma, Véronique Kalck, Kazumi Akimura, Angela Taddei, Hitoshi Iwahashi, Kazuto Kugou, Kunihiro Ohta, Susan M Gasser, Masahiko Harata (2010 Apr 27)

Actin-related protein Arp6 influences H2A.Z-dependent and -independent gene expression and links ribosomal protein genes to nuclear pores.

PLoS genetics : e1000910 : [DOI : 10.1371/journal.pgen.1000910](https://doi.org/10.1371/journal.pgen.1000910)

Résumé

Actin-related proteins are ubiquitous components of chromatin remodelers and are conserved from yeast to man. We have examined the role of the budding yeast actin-related protein Arp6 in gene expression, both as a component of the SWR1 complex (SWR-C) and in its absence. We mapped Arp6 binding sites along four yeast chromosomes using chromatin immunoprecipitation from wild-type and *swr1* deleted (*swr1Delta*) cells. We find that a majority of Arp6 binding sites coincide with binding sites of Swr1, the catalytic subunit of SWR-C, and with the histone H2A variant Htz1 (H2A.Z) deposited by SWR-C. However, Arp6 binding detected at centromeres, the promoters of ribosomal protein (RP) genes, and some

Compartmentation et dynamique des fonctions nucléaires

telomeres is independent of Swr1 and Htz1 deposition. Given that RP genes and telomeres both show association with the nuclear periphery, we monitored the ability of Arp6 to mediate the localization of chromatin to nuclear pores. Arp6 binding is sufficient to shift a randomly positioned locus to nuclear periphery, even in a *swr1Delta* strain. Arp6 is also necessary for the pore association of its targeted RP promoters possibly through cell cycle-dependent factors. Loss of Arp6, but not Htz1, leads to an up-regulation of these RP genes. In contrast, the pore-association of GAL1 correlates with Htz1 deposition, and loss of Arp6 reduces both GAL1 activation and peripheral localization. We conclude that Arp6 functions both together with the nucleosome remodeler Swr1 and also without it, to mediate Htz1-dependent and Htz1-independent binding of chromatin domains to nuclear pores. This association is shown to have modulating effects on gene expression.