Compartmentalization and Dynamics of Nuclear Functions

Ophélie Lautier, Arianna Penzo, Jérôme O Rouvière, Guillaume Chevreux, Louis Collet, Isabelle Loiodice, Angela Taddei, Frédéric Devaux, Martine A Collart, Benoit Palancade (2021 Apr 10)

Co-translational assembly and localized translation of nucleoporins in nuclear pore complex biogenesis.

Molecular cell: DOI : S1097-2765(21)00225-2

Summary

mRNA translation is coupled to multiprotein complex assembly in the cytoplasm or to protein delivery into intracellular compartments. Here, by combining systematic RNA immunoprecipitation and single-molecule RNA imaging in yeast, we have provided a complete depiction of the co-translational events involved in the biogenesis of a large multiprotein assembly, the nuclear pore complex (NPC). We report that binary interactions between NPC subunits can be established during translation, in the cytoplasm. Strikingly, the nucleoporins Nup1/Nup2, together with a number of nuclear proteins, are instead translated at nuclear pores, through a mechanism involving interactions between their nascent N-termini and nuclear transport receptors. Uncoupling this co-translational recruitment further triggers the formation of cytoplasmic foci of unassembled polypeptides. Altogether, our data reveal that distinct, spatially segregated modes of co-translational interactions foster the ordered assembly of NPC subunits and that localized translation can ensure the proper delivery of proteins to the pore and the nucleus.

Heltberg Mathias, Miné-Hattab Judith, Taddei Angela, Walczak Aleksandra M., Mora Thierry (2021 Apr 2)

Physical observables to determine the nature of membrane-less cellular sub-compartments

preprint. : DOI : 10.1101/2021.04.01.438041

Summary

Abstract

The spatial organization of complex biochemical reactions is essential for the regulation of cellular processes. Membrane-less structures called foci containing high concentrations of specific proteins have been reported in a variety of contexts, but the mechanism of their formation is not fully understood. Several competing mechanisms exist that are difficult to distinguish empirically, including liquid-liquid phase separation, and the trapping of molecules by multiple binding sites. Here we propose a theoretical framework and outline observables to differentiate between these scenarios from single molecule tracking experiments. In the binding site model, we derive relations between the distribution of proteins, their diffusion properties, and their radial displacement. We predict that protein
search times can be reduced for targets inside a liquid droplet, but not in an aggregate of slowly moving binding sites. These results are applicable to future experiments and suggest different biological roles for liquid droplet and binding site foci.

Myriam Ruault, Vittore F Scolari, Luciana Lazar-Stefanita, Antoine Hocher, Isabelle Loiodice, Romain Koszul, Angela Taddei (2021 Feb 13)
**Sir3 mediates long-range chromosome interactions in budding yeast.**
*Genome research* : 411-425 : [DOI : 10.1101/gr.267872.120](https://doi.org/10.1101/gr.267872.120)

**Summary**

Physical contacts between distant loci contribute to regulate genome function. However, the molecular mechanisms responsible for settling and maintaining such interactions remain poorly understood. Here, we investigate the well-conserved interactions between heterochromatin loci. In budding yeast, the 32 telomeres cluster in 3-5 foci in exponentially growing cells. This clustering is functionally linked to the formation of heterochromatin in subtelomeric regions through the recruitment of the silencing SIR complex composed of Sir2/3/4. Combining microscopy and Hi-C on strains expressing different alleles of , we show that the binding of Sir3 directly promotes long-range contacts between distant regions, including the rDNA, telomeres, and internal Sir3-bound sites. Furthermore, we unveil a new property of Sir3 in promoting rDNA compaction. Finally, using a synthetic approach, we demonstrate that Sir3 can bond loci belonging to different chromosomes together, when targeted to these loci, independently of its interaction with its known partners (Rap1, Sir4), Sir2 activity, or chromosome context. Altogether, these data suggest that Sir3 acts as a molecular bridge that stabilizes long-range interactions.

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](https://doi.org/10.7554/eLife.60577)

**Summary**

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.

Year of publication 2020

Judith Miné-hattab, Irène Chiolo (2020 Aug 27)
**Complex Chromatin Motions for DNA Repair**

**Summary**

A number of studies across different model systems revealed that chromatin undergoes significant changes in dynamics in response to DNA damage. These include local motion changes at damage sites, increased nuclear exploration of both damaged and undamaged loci, and directed motions to new nuclear locations associated with certain repair pathways. These studies also revealed the need for new analytical methods to identify directed motions in a context of mixed trajectories, and the importance of investigating nuclear dynamics over different time scales to identify diffusion regimes. Here we provide an overview of the current understanding of this field, including imaging and analytical methods developed to investigate nuclear dynamics in different contexts. These dynamics are essential for genome integrity. Identifying the molecular mechanisms responsible for these movements is key to understanding how their misregulation contributes to cancer and other genome instability disorders.

HOCHER Antoine, TADDEI Angela (2020 Mar 17)
**Subtelomeres as Specialized Chromatin Domains**
*Bioessays review*: DOI: [10.1002/bies.201900205](10.1002/bies.201900205)

**Summary**

Year of publication 2019

Garance Alberman, Jean-Marie Gagez, Judith Miné-Hattab (2019 Dec 18)
[When science and music meet].
*Medecine sciences*: DOI: [10.1051/medsci/2019169](10.1051/medsci/2019169)

**Summary**

Elie Dolgin (2019 May 7)
**The sounds of science: biochemistry and the cosmos inspire new music**
*Nature*: DOI: [10.1038/d41586-019-01422-0](10.1038/d41586-019-01422-0)
Summary

Judith Miné-Hattab, Angela Taddei (2019 Apr 1)
Physical principles and functional consequences of nuclear compartmentalization in budding yeast.
Current opinion in cell biology : 105-113 : DOI : 10.1016/j.ceb.2019.02.005

Summary

One striking feature of eukaryotic nuclei is the existence of discrete regions, in which specific factors concentrate while others are excluded, thus forming microenvironments with different molecular compositions and biological functions. These domains are often referred to as subcompartments even though they are not membrane enclosed. Despite their functional importance the physical nature of these structures remains largely unknown. Here, we describe how the Saccharomyces cerevisiae nucleus is compartmentalized and discuss possible physical models underlying the formation and maintenance of chromatin associated subcompartments. Focusing on three particular examples, the nucleolus, silencing foci, and repair foci, we discuss the biological implications of these different models as well as possible approaches to challenge them in living cells.

Klein et al. (2019 Jan 7)
Guidelines for DNA recombination and repair studies: Cellular assays of DNA repair pathways
Microb Cell- : DOI : 10.15698/mic2019.01.664

Summary

Year of publication 2018

Judith Miné-Hattab, Xavier Darzacq (2018 Nov 20)
[Chromatin mobility upon DNA damage: a multi-scale story].
Medecine sciences : M/S : 778-781 : DOI : 10.1051/medsci/2018214

Summary

Antoine Hocher, Myriam Ruault, Petra Kaferle, Marc Descrimes, 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

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Summary

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.

Year of publication 2017

Multi-scale tracking reveals scale-dependent chromatin dynamics after DNA damage.
Molecular biology of the cell: DOI: mbc.E17-05-0317

Summary

The dynamic organization of genes inside the nucleus is an important determinant for their function. Using fast DNA tracking microscopy in cells and improved analysis of mean square displacements, we quantified DNA motion at time scales ranging from 10 milliseconds to minute and found that following DNA damage, DNA exhibits distinct sub-diffusive regimes. In response to double-strand breaks, chromatin is more mobile at large time scales but, surprisingly, its mobility is reduced at short time scales. This effect is even more pronounced at the site of damage. Such a pattern of dynamics is consistent with a global increase in chromatin persistence length in response to DNA damage. Scale-dependent nuclear exploration is regulated by the Rad51 repair protein, both at the break and throughout the genome. We propose a model in which stiffening of the damaged ends by the repair complex, combined with global increased stiffness, act like a “needle in a ball of yarn”, enhancing the ability of the break to traverse the chromatin meshwork.

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

Summary

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

Summary