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Żylicz Jan Jakub, Bousard Aurélie, Žumer Kristina, Dossin François, Mohammad Eusra, Teixeira da Rocha Simão, Schwalb Björn, Syx Laurène, Dingli Florent, Loew Damarys, Cramer Patrick, Heard Edith (2018 Dec 21)

The Implication of Early Chromatin Changes in X Chromosome Inactivation

Cell : 176 : 1-16 : [DOI : 10.1016/j.cell.2018.11.041](https://doi.org/10.1016/j.cell.2018.11.041)

Résumé

During development, the precise relationships between transcription and chromatin modifications often remain unclear. We use the X chromosome inactivation (XCI) paradigm to explore the implication of chromatin changes in gene silencing. Using female mouse embryonic stem cells, we initiate XCI by inducing Xist and then monitor the temporal changes in transcription and chromatin by allele-specific profiling. This reveals histone deacetylation and H2AK119 ubiquitination as the earliest chromatin alterations during XCI. We show that HDAC3 is pre-bound on the X chromosome and that, upon Xist coating, its activity is required for efficient gene silencing. We also reveal that first PRC1-associated H2AK119Ub and then PRC2-associated H3K27me3 accumulate initially at large intergenic domains that can then spread into genes only in the context of histone deacetylation and gene silencing. Our results reveal the hierarchy of chromatin events during the initiation of XCI and identify key roles for chromatin in the early steps of transcriptional silencing.

Année de publication : 2017

Simão T da Rocha, Edith Heard (2017 Mar 4)

Novel players in X inactivation: insights into Xist-mediated gene silencing and chromosome conformation.

Nature structural & molecular biology : 197-204 : [DOI : 10.1038/nsmb.3370](https://doi.org/10.1038/nsmb.3370)

Résumé

The nuclear long noncoding RNA (lncRNA) Xist ensures X-chromosome inactivation (XCI) in female placental mammals. Although Xist is one of the most intensively studied lncRNAs, the mechanisms associated with its capacity to trigger chromosome-wide gene silencing, the formation of facultative heterochromatin and an unusual 3D conformation of the inactive X chromosome (Xi) have remained elusive. Now researchers have identified novel functional partners of Xist in a series of breakthrough studies, using unbiased techniques to isolate Xist-bound proteins, as well as forward genetic screens. In addition, important insights into the 3D organization of Xi and its relation to gene expression have been obtained. In this Review, we discuss how this new information is providing a recipe for deciphering XCI mechanisms by which a multitasking RNA can structurally and functionally transform an active chromosome into uniquely organized facultative heterochromatin.

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

Maud Borensztein, Laurène Syx, Katia Ancelin, Patricia Diabangouaya, Christel Picard, Tao Liu, Jun-Bin Liang, Ivaylo Vassilev, Rafael Galupa, Nicolas Servant, Emmanuel Barillot, Azim Surani, Chong-jian Chen, Edith Heard (2017 Jan 31)

Xist-dependent imprinted X inactivation and the early developmental consequences of its failure.

Nature structural & molecular biology : [DOI : 10.1038/nsmb.3365](https://doi.org/10.1038/nsmb.3365)

Résumé

The long noncoding RNA Xist is expressed from only the paternal X chromosome in mouse preimplantation female embryos and mediates transcriptional silencing of that chromosome. In females, absence of Xist leads to postimplantation lethality. Here, through single-cell RNA sequencing of early preimplantation mouse embryos, we found that the initiation of imprinted X-chromosome inactivation absolutely requires Xist. Lack of paternal Xist leads to genome-wide transcriptional misregulation in the early blastocyst and to failure to activate the extraembryonic pathway that is essential for postimplantation development. We also demonstrate that the expression dynamics of X-linked genes depends on the strain and parent of origin as well as on the location along the X chromosome, particularly at the first 'entry' sites of Xist. This study demonstrates that dosage-compensation failure has an effect as early as the blastocyst stage and reveals genetic and epigenetic contributions to orchestrating transcriptional silencing of the X chromosome during early embryogenesis.

Jin Xu, Ava C Carter, Anne-Valerie Gendrel, Mikael Attia, Joshua Loftus, William J Greenleaf, Robert Tibshirani, Edith Heard, Howard Y Chang (2017 Jan 24)

Landscape of monoallelic DNA accessibility in mouse embryonic stem cells and neural progenitor cells.

Nature genetics : [DOI : 10.1038/ng.3769](https://doi.org/10.1038/ng.3769)

Résumé

We developed an allele-specific assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq) to genotype and profile active regulatory DNA across the genome. Using a mouse hybrid F1 system, we found that monoallelic DNA accessibility across autosomes was pervasive, developmentally programmed and composed of several patterns. Genetically determined accessibility was enriched at distal enhancers, but random monoallelically accessible (RAMA) elements were enriched at promoters and may act as gatekeepers of monoallelic mRNA expression. Allelic choice at RAMA elements was stable

across cell generations and bookmarked through mitosis. RAMA elements in neural progenitor cells were biallelically accessible in embryonic stem cells but premarked with bivalent histone modifications; one allele was silenced during differentiation. Quantitative analysis indicated that allelic choice at the majority of RAMA elements is consistent with a stochastic process; however, up to 30% of RAMA elements may deviate from the expected pattern, suggesting a regulated or counting mechanism.

Yinxu Zhan, Luca Mariani, Iros Barozzi, Edda G Schulz, Nils Blüthgen, Michael Stadler, Guido Tiana, Luca Giorgetti (2017 Jan 7)

Reciprocal insulation analysis of Hi-C data shows that TADs represent a functionally but not structurally privileged scale in the hierarchical folding of chromosomes.

Genome research : 479-490 : [DOI : 10.1101/gr.212803.116](https://doi.org/10.1101/gr.212803.116)

Résumé

Understanding how regulatory sequences interact in the context of chromosomal architecture is a central challenge in biology. Chromosome conformation capture revealed that mammalian chromosomes possess a rich hierarchy of structural layers, from multi-megabase compartments to sub-megabase topologically associating domains (TADs) and sub-TAD contact domains. TADs appear to act as regulatory microenvironments by constraining and segregating regulatory interactions across discrete chromosomal regions. However, it is unclear whether other (or all) folding layers share similar properties, or rather TADs constitute a privileged folding scale with maximal impact on the organization of regulatory interactions. Here, we present a novel algorithm named CaTCH that identifies hierarchical trees of chromosomal domains in Hi-C maps, stratified through their reciprocal physical insulation, which is a single and biologically relevant parameter. By applying CaTCH to published Hi-C data sets, we show that previously reported folding layers appear at different insulation levels. We demonstrate that although no structurally privileged folding level exists, TADs emerge as a functionally privileged scale defined by maximal boundary enrichment in CTCF and maximal cell-type conservation. By measuring transcriptional output in embryonic stem cells and neural precursor cells, we show that the likelihood that genes in a domain are coregulated during differentiation is also maximized at the scale of TADs. Finally, we observe that regulatory sequences occur at genomic locations corresponding to optimized mutual interactions at the same scale. Our analysis suggests that the architectural functionality of TADs arises from the interplay between their ability to partition interactions and the specific genomic position of regulatory sequences.

Année de publication : 2016

Edith Heard (2016 Nov 22)

3D solutions to complex gene regulation.

Nature reviews. Molecular cell biology : 739 : [DOI : 10.1038/nrm.2016.154](https://doi.org/10.1038/nrm.2016.154)

Résumé

Luca Giorgetti, Edith Heard (2016 Oct 21)

Closing the loop: 3C versus DNA FISH.

Genome biology : 215

Résumé

Chromosome conformation capture (3C)-based techniques have revolutionized the field of nuclear organization, partly replacing DNA FISH as the method of choice for studying three-dimensional chromosome architecture. Although DNA FISH is commonly used for confirming 3C-based findings, the two techniques are conceptually and technically different and comparing their results is not trivial. Here, we discuss both 3C-based techniques and DNA FISH approaches to highlight their similarities and differences. We then describe the technical biases that affect each approach, and review the available reports that address their compatibility. Finally, we propose an experimental scheme for comparison of 3C and DNA FISH results.

Catherine Corbel, Edith Heard (2016 Sep 30)

Transcriptional Analysis by Nascent RNA FISH of In Vivo Trophoblast Giant Cells or In Vitro Short-term Cultures of Ectoplacental Cone Explants.

Journal of visualized experiments : JoVE : [DOI : 10.3791/54386](https://doi.org/10.3791/54386)

Résumé

The placenta derives from one extra-embryonic lineage, the trophoblast. In the peri-implantation murine blastocyst, mural trophoblast cells differentiate into primary trophoblast giant cells (TGCs) while the polar trophoblast overlying the inner cell mass continues to proliferate later differentiating into secondary TGCs. TGCs play a key role in developing placenta and are essential for a successful pregnancy. Investigation of transcriptional regulation of specific genes during post-implantation development can give insights into TGCs development. Cells of the ectoplacental cone (EPC) from embryos at 7-7.5 days of gestation (E7-7.5), derived from the polar trophoblast, differentiate into secondary TGCs(1). TGCs can be studied in situ, on cryostat sections of embryos at E7 although the number of TGCs is very low at this stage. An alternative means of analyzing secondary TGCs is to use short-term cultures of individual EPCs from E7 embryos. We propose a technique to investigate the transcriptional status of genes of interest both in vivo and in vitro at the single-cell level using fluorescent in situ hybridization (RNA FISH) to visualize nascent transcripts. This technique provides a direct readout of gene expression and enables assessment of the chromosomal status of TGCs, which are large endoreplicating cells. Indeed, a key feature of terminal differentiation of TGCs is that they exit the cell cycle and undergo multiple rounds of endoreplication. This approach can be applied to detect expression of any gene expressed from autosomes and/or sex chromosomes and can provide important information into developmental mechanisms as well as placental diseases.

Luca Giorgetti, Bryan R Lajoie, Ava C Carter, Mikael Attia, Ye Zhan, Jin Xu, Chong Jian Chen, Noam Kaplan, Howard Y Chang, Edith Heard, Job Dekker (2016 Jul 21)

Structural organization of the inactive X chromosome in the mouse.

Nature : DOI : [10.1038/nature18589](https://doi.org/10.1038/nature18589)

Résumé

X-chromosome inactivation (XCI) involves major reorganization of the X chromosome as it becomes silent and heterochromatic. During female mammalian development, XCI is triggered by upregulation of the non-coding Xist RNA from one of the two X chromosomes. Xist coats the chromosome in cis and induces silencing of almost all genes via its A-repeat region, although some genes (constitutive escapees) avoid silencing in most cell types, and others (facultative escapees) escape XCI only in specific contexts. A role for Xist in organizing the inactive X (Xi) chromosome has been proposed. Recent chromosome conformation capture approaches have revealed global loss of local structure on the Xi chromosome and formation of large mega-domains, separated by a region containing the DXZ4 macrosatellite. However, the molecular architecture of the Xi chromosome, in both the silent and expressed regions, remains unclear. Here we investigate the structure, chromatin accessibility and expression status of the mouse Xi chromosome in highly polymorphic clonal neural progenitors (NPCs) and embryonic stem cells. We demonstrate a crucial role for Xist and the DXZ4-containing boundary in shaping Xi chromosome structure using allele-specific genome-wide chromosome conformation capture (Hi-C) analysis, an assay for transposase-accessible chromatin with high throughput sequencing (ATAC-seq) and RNA sequencing. Deletion of the boundary disrupts mega-domain formation, and induction of Xist RNA initiates formation of the boundary and the loss of DNA accessibility. We also show that in NPCs, the Xi chromosome lacks active/inactive compartments and topologically associating domains (TADs), except around genes that escape XCI. Escapee gene clusters display TAD-like structures and retain DNA accessibility at promoter-proximal and CTCF-binding sites. Furthermore, altered patterns of facultative escape genes in different neural progenitor clones are associated with the presence of different TAD-like structures after XCI. These findings suggest a key role for transcription and CTCF in the formation of TADs in the context of the Xi chromosome in neural progenitors.

Anthony Ferrari, Anne Vincent-Salomon, Xavier Pivot, Anne-Sophie Sertier, Emilie Thomas, Laurie Tonon, Sandrine Boyault, Eskeatnaf Mulugeta, Isabelle Treilleux, Gaëtan MacGrogan, Laurent Arnould, Janice Kielbassa, Vincent Le Texier, Hélène Blanché, Jean-François Deleuze, Jocelyne Jacquemier, Marie-Christine Mathieu, Frédérique Penault-Llorca, Frédéric Bibeau, Odette Mariani, Cécile Mannina, Jean-Yves Pierga, Olivier Trédan, Thomas Bachelot, Hervé Bonnefoi, Gilles Romieu, Pierre Fumoleau, Suzette Delalogue, Maria Rios, Jean-Marc Ferrero, Carole Tarpin, Catherine Bouteille, Fabien Calvo, Ivo Glynne Gut, Marta Gut, Sancha Martin, Serena Nik-Zainal, Michael R Stratton, Iris Pauporté, Pierre Saintigny, Daniel Birnbaum, Alain Viari, Gilles Thomas (2016 Jul 14)

A whole-genome sequence and transcriptome perspective on HER2-positive

breast cancers.

Nature communications : 12222 : [DOI : 10.1038/ncomms12222](https://doi.org/10.1038/ncomms12222)

Résumé

HER2-positive breast cancer has long proven to be a clinically distinct class of breast cancers for which several targeted therapies are now available. However, resistance to the treatment associated with specific gene expressions or mutations has been observed, revealing the underlying diversity of these cancers. Therefore, understanding the full extent of the HER2-positive disease heterogeneity still remains challenging. Here we carry out an in-depth genomic characterization of 64 HER2-positive breast tumour genomes that exhibit four subgroups, based on the expression data, with distinctive genomic features in terms of somatic mutations, copy-number changes or structural variations. The results suggest that, despite being clinically defined by a specific gene amplification, HER2-positive tumours melt into the whole luminal-basal breast cancer spectrum rather than standing apart. The results also lead to a refined ERBB2 amplicon of 106 kb and show that several cases of amplifications are compatible with a breakage-fusion-bridge mechanism.

Anne-Valerie Gendrel, Lucile Marion-Poll, Kimiko Katoh, Edith Heard (2016 Apr 23)

Random monoallelic expression of genes on autosomes: Parallels with X-chromosome inactivation.

Seminars in cell & developmental biology : [DOI : S1084-9521\(16\)30102-1](https://doi.org/10.1016/j.sembio.2016.03.001)

Résumé

Genes are generally expressed from their two alleles, except in some particular cases such as random inactivation of one of the two X chromosomes in female mammals or imprinted genes which are expressed only from the maternal or the paternal allele. A lesser-known phenomenon is random monoallelic expression (RME) of autosomal genes, where genes can be stably expressed in a monoallelic manner, from either one of the parental alleles. Studies on autosomal RME face several challenges. First, RME that is based on epigenetic mechanisms has to be distinguished from biased expression of one allele caused by a DNA sequence polymorphism in a regulatory element. Second, RME should not be confused with transient monoallelic expression often observed in single cell analyses, and that often corresponds to dynamic bursting of expression. Thanks to analyses on clonal cell populations, the existence of RME in cultured cells is now well established. Future studies of RME in vivo will have to overcome tissue heterogeneity and certain technical limitations. Here, we discuss current knowledge on autosomal RME, as well as possible mechanisms controlling these expression patterns and potential implications for development and disease, drawing parallels with what is known for X-chromosome inactivation, a paradigm of random monoallelic expression.

Guido Tiana, Assaf Amitai, Tim Pollex, Tristan Pilot, David Holcman, Edith Heard, Luca Giorgetti (2016 Mar 31)

Structural Fluctuations of the Chromatin Fiber within Topologically Associating Domains.

Biophysical journal : 1234-45 : [DOI : 10.1016/j.bpj.2016.02.003](https://doi.org/10.1016/j.bpj.2016.02.003)

Résumé

Experiments based on chromosome conformation capture have shown that mammalian genomes are partitioned into topologically associating domains (TADs), within which the chromatin fiber preferentially interacts. TADs may provide three-dimensional scaffolds allowing genes to contact their appropriate distal regulatory DNA sequences (e.g., enhancers) and thus to be properly regulated. Understanding the cell-to-cell and temporal variability of the chromatin fiber within TADs, and what determines them, is thus of great importance to better understand transcriptional regulation. We recently described an equilibrium polymer model that can accurately predict cell-to-cell variation of chromosome conformation within single TADs, from chromosome conformation capture-based data. Here we further analyze the conformational and energetic properties of our model. We show that the chromatin fiber within TADs can easily fluctuate between several conformational states, which are hierarchically organized and are not separated by important free energy barriers, and that this is facilitated by the fact that the chromatin fiber within TADs is close to the onset of the coil-globule transition. We further show that in this dynamic state the properties of the chromatin fiber, and its contact probabilities in particular, are determined in a nontrivial manner not only by site-specific interactions between strongly interacting loci along the fiber, but also by nonlocal correlations between pairs of contacts. Finally, we use live-cell experiments to measure the dynamics of the chromatin fiber in mouse embryonic stem cells, in combination with dynamical simulations, and predict that conformational changes within one TAD are likely to occur on timescales that are much shorter than the duration of one cell cycle. This suggests that genes and their regulatory elements may come together and disassociate several times during a cell cycle. These results have important implications for transcriptional regulation as they support the concept of highly dynamic interactions driven by a complex interplay between site-specific interactions and the intrinsic biophysical properties of the chromatin fiber.

Katia Ancelin, Laurène Syx, Maud Borensztein, Noémie Ranisavljevic, Ivaylo Vassilev, Luis Briseño-Roa, Tao Liu, Eric Metzger, Nicolas Servant, Emmanuel Barillot, Chong-Jian Chen, Roland Schüle, Edith Heard (2016 Feb 3)

Maternal LSD1/KDM1A is an essential regulator of chromatin and transcription landscapes during zygotic genome activation.

eLife : [DOI : 10.7554/eLife.08851](https://doi.org/10.7554/eLife.08851)

Résumé

Upon fertilization, the highly specialised sperm and oocyte genomes are remodelled to confer totipotency. The mechanisms of the dramatic reprogramming events that occur have remained unknown, and presumed roles of histone modifying enzymes are just starting to be elucidated. Here, we explore the function of the oocyte-inherited pool of a histone H3K4 and

K9 demethylase, LSD1/KDM1A during early mouse development. KDM1A deficiency results in developmental arrest by the two-cell stage, accompanied by dramatic and stepwise alterations in H3K9 and H3K4 methylation patterns. At the transcriptional level, the switch of the maternal-to-zygotic transition fails to be induced properly and LINE-1 retrotransposons are not properly silenced. We propose that KDM1A plays critical roles in establishing the correct epigenetic landscape of the zygote upon fertilization, in preserving genome integrity and in initiating new patterns of genome expression that drive early mouse development.

Année de publication : 2015

Job Dekker, Edith Heard (2015 Sep 9)

Structural and functional diversity of Topologically Associating Domains.

FEBS letters : 2877-84 : [DOI : 10.1016/j.febslet.2015.08.044](https://doi.org/10.1016/j.febslet.2015.08.044)

Résumé

Recent studies have shown that chromosomes in a range of organisms are compartmentalized in different types of chromatin domains. In mammals, chromosomes form compartments that are composed of smaller Topologically Associating Domains (TADs). TADs are thought to represent functional domains of gene regulation but much is still unknown about the mechanisms of their formation and how they exert their regulatory effect on embedded genes. Further, similar domains have been detected in other organisms, including flies, worms, fungi and bacteria. Although in all these cases these domains appear similar as detected by 3C-based methods, their biology appears to be quite distinct with differences in the protein complexes involved in their formation and differences in their internal organization. Here we outline our current understanding of such domains in different organisms and their roles in gene regulation.