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

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.
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

Résumé

The placenta derives from one extra-embryonic lineage, the trophectoderm. In the peri-implantation murine blastocyst, mural trophectoderm cells differentiate into primary trophoblast giant cells (TGCs) while the polar trophectoderm 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 trophectoderm, 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.