Talk

Impact of the expression of human CTCF protein in 3D organization of the Saccharomyces cerevisiae genome

Inmaculada Vázquez Gutiérrez, Andrés Garzón Villar, Ignacio Maeso Martín

Centro Andaluz de Biología del Desarrollo (CABD), Carretera de Utrera Km 1 41013 Sevilla

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ABSTRACT

Motivation: Transcriptional regulation is particularly complex in animals and depends on long-range interactions between multiple distal enhancers and their target promoters. Thus, the 3D organization of the chromatin is critical to guarantee this interactions and to avoid the spurious ones. In different groups of animals, such as humans and other vertebrates, the protein CTCF works as an essential factor to control the 3D structure of the genome, regulating cohesin-mediated chromatin interactions and the formation of loops between distal enhancers and their target promoters. In contrast, this type of long-range cis-regulation and its associated 3D chromatin organization have not been observed in other eukaryotic lineages such as plants and fungi. Interestingly, CTCF is also absent from the genome of these non-animal species. To investigate how the origin of CTCF could have contributed to the evolution of long-range chromatin interactions in animals, we have used the model organism Saccharomyces cerevisiae to study the effects that the expression of CTCF may have on the 3D organization of a fungal genome that does not have distal cis-regulation.

Methods: We are generating two different yeast strains. Both of them contain a plasmid expressing human CTCF under the control of the inducible galactose promoter, but one of the strains will be further modified by the introduction of a sequence containing binding sites for human CTCF through homologous recombination using sigma LTR sequences from the Ty3 retrotransposon.

Results: We have already generated a yeast strain to express human CTCF, and this strain is able to survive when we induce CTCF expression with galactose. Furthermore, to confirm the expression of CTCF we have done a western blot. Finally, we have designed the sigma Ty3 construct to introduce CTCF binding sites by selecting a human DNA sequence associated to chromatin loop border.

Conclusions: We have confirmed that S.cerevisiae is still viable when it expresses the CTCF protein. The CTCF protein. The next steps will be i) to synthetize the sigma Ty3 construct with CTCF sites and introduce it in the yeast genome, ii) to analize by ChIP-seq if CTCF is able to bind endogenous yeast regions or if it can only bind those sites introduced using the sigma Ty3 construct and iii) to study how the presence of CTCF with and without the insertion of human CTCF binding sites affects the 3D chromatin organization of S.cerevisiae.

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