DYNAMICS AND SUPRANUCLEOSOMAL ORGANIZATION OF THE MAMMALIAN CHROMATIN : A CONTRIBUTION OF THE 3C-qPCR METHOD

Franck COURT, Julie MIRO, Caroline BRAEM, Marie-Noëlle LE LAY-TAHA, Audrey BRISEBARRE, Florian ATGER, Thierry GOSTAN, Michael WEBER, Guy CATHALA, and Thierry FORNE

Institut de Génétique Moléculaire de Montpellier, UMR 5535 CNRS-UMII, 1919 Route de Mende, 34293 Montpellier cedex 5, France.

In interphasic cells, the mammalian genome, packed into the chromatin, is spatially restrained to specific chromosomal territories. However, beyond the simple nucleosomal array, very little is known about the organization and the dynamics of the chromatin within chromosomal territories. Although it is largely admitted that one essential determinant is chromatin-looping in relation with gene expression and other chromosomal activities, the basic structural landscape of the chromatin remains largely unknown at the supranucleosomal level (~10-350 kb). The Chromosome Conformation Capture (3C) assay, and derived technologies, represents a real technological advance. Indeed, such techniques allow direct quantification of the average interaction frequency between two distant genomic regions, at the supranucleosomal scale, in their native genomic context.

Our laboratory contributed to the recent development of the 3C technologies by improving the quantification of the interaction frequencies (3C-qPCR method) (ref.1).

- In a first approach, we applied this method to analyze long-range chromatin interactions at the *Dlk1/Gtl2* (ref.2) and *Igf2/H19* imprinted loci. We identified several locus-specific looping interactions, thus bringing original insights into the complex mechanisms of gene regulation at both loci.
- In a second approach, we have determined random collision frequencies occurring between sites separated by increasing genomic distances and sought to establish whether some fundamental intrinsic constraints apply to the supranucleosomal chromatin. We demonstrate that, at several gene-rich loci where no long-range locus-specific interactions could be detected, the collision frequencies display a basic statistical oscillation that periodically modulates (*i.e.* every 90-100kb) their dynamics over large genomic distances. Such an oscillation is not found at a gene-desert locus.

Using HI-C data from Job Dekker laboratory (ref.3), we found that long-range interactions in Giemsa-negative bands (gene-rich regions) are favoured for site separations around 100kb relative to Giemsa-positive bands (gene-poor regions). Further bioinformatics analyses showed that conserved sequences at co-regulated gene loci are highly overrepresented at genomic distances corresponding to the first and second modulations relative to transcription start sites. Therefore, the basic 90-100kb oscillation revealed in our experiments may underlie the dynamics of a significant part of long-range interactions thus contributing to mammalian genome evolution. Finally, we show that the dynamics of random collisions observed at gene-rich loci can be described by polymer models as if the chromatin was shaped into a statistical helix.

1- Hagège *et al.*, 2007 *Nat. Protocols* 2, 1722
2- Braem *et al.*, 2008 *J. Biol. Chem.* 283, 18612
3- Lieberman-Aiden *et al.*, 2009 *Sciences* 326, 289