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Review

Can chromatin conformation technologies bring light into human molecular pathology?

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Regulation of gene expression in eukaryotes involves many complex processes, in which chromatin structure plays an important role. In addition to the epigenetic effects, such as DNA methylation and phosphorylation or histone modifications, gene expression is also controlled by the spatial organization of chromatin. For example, distant regulatory elements (enhancers, insulators) may come into direct physical interaction with target genes or other regulatory elements located in genomic regions of up to several hundred kilobases in size. Such long-range interactions result in the formation of chromatin loops. In the last several years, there has been a rapid increase in our knowledge of the spatial organization of chromatin in the nucleus through the chromosome conformation capture (3C) technology. Here we review and compare the original 3C and 3C-based methods including chromosome conformation capture-on-chip (4C), chromosome conformation capture carbon copy (5C), hi-resolution chromosome confomation capture (HiC). In this article, we discuss different aspects of how the nuclear organization of chromatin is associated with gene expression regulation and how this knowledge is useful in translational medicine and clinical applications. We demonstrate that the knowledge of the chromatin 3D organization may help understand the mechanisms of gene expression regulation of genes involved in the development of human diseases, such as *CFTR* (responsible for cystic fibrosis) or *IGFBP3* (associated with breast cancer pathogenesis). Additionally, 3C-derivative methods have been also useful in the diagnosis of some leukemia subtypes.

Key words: chromosome conformation capture, human molecular pathology, chromatin, chromatin looping, genome organization

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INTRODUCTION

Interactions between different chromosomal regions play an important role in the mechanism of gene expression regulation (Rodriguez & Bjerling, 2013). In the last few years, the knowledge of the spatial organization of chromatin inside the nucleus has been expanded thanks to the newly developed technology called chromosome conformation capture (3C) and its derivative methods, such as chromosome conformation capture-on-chip (4C), chromosome conformation capture carbon copy (5C) and hi-resolution chromosome confomation capture (HiC), Targeted Chromatin Capture (T2C) and Capture C. 3C technology and its genome-wide applications (4C, 5C, HiC, T2C, Capture C) have identified many distal DNA sequences like regulatory elements (enhancers, insulators) that are frequently in close spatial proximity to promoters of their genes. Such long-range interactions result in the formation of chromatin loops and have been correlated with mechanisms of transcriptional regulation of the interacting genes. To better understand how the 3D architecture of chromatin is associated with mechanism of gene expression regulation, changes in chromatin organization must be correlated with other epigenetic effects, such as binding of transcription factors, architectural proteins or histone modifications (Sexton *et al*., 2009). Chromatin conformation capture and its derivative methods start with endpoint PCR and real-time PCR (3C), and range to high throughput and high resolution methods like microarray (4C) or next generation sequencing (5C, HiC, T2C, Capture C). All of these techniques have their own advantages and limitations (Table 1) and have provided very valuable information on chromosomal interactions and gene transcription mechanisms.

One of the first methods used for investigating the spatial organization of chromatin was the RNA-TRAP technique (tagging and recovery of associated proteins) (Carter *et al*., 2002). The RNA-TRAP method was based on the targeting of peroxidase activity to transcripts and its use was restricted to indicating physical interactions of chromatin in actively transcribed genes (Carter *et al*., 2002). A novel chromosome conformation capture (3C) methodology allows the exploration of the 3D organization of chromatin at any selected genomic site of interest. Furthermore, the 4C, 5C and HiC technologies allow the investigation of chromatin architecture within the entire genome.

CHROMOSOME CONFORMATION CAPTURE (3C)

Chromosome conformation capture was developed more than 10 years ago by Dekker and coworkers (Dekker *et al*., 2002) and, since then, has been used to study the spatial organization of chromatin in living cells. The 3C method allows the identification of chromatin loops in a single locus or chromatin subdomains formed in genomic regions of up to several hundred kilobases in size (Hagege *et al*., 2007). This method allows studies of the mechanism of gene regulation involving the

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Abbreviations: 3C, chromatin conformation capture; 4C, chromosome conformation capture-on-chip; 5C, chromosome conformation capture carbon copy; ChIP, chromatin immunoprecipitation; HiC, high resolution chromosome confomation capture, NGS, next-generation sequencing; OCT, oligonucleotide capture technology; RNA-TRAP, tagging and recovery of associated proteins; T2C, Targeted Chromatin Capture

Table 1. Comparison between the presented 3C and 3C-derivative technologies.

Quality analysis	Method	Advantages	Limitations
One *vs* One	3C	Simple analysis, low cost	Knowledge of the targeted sequences, unsuitable for the analysis of long-range contacts, low resolution
One *vs* all	4C	High resolution	Genome wide but anchored on single locus, difficult anaylsis
Many *vs* Many	5C	Parallel analysis of interactions between many sites of interest	Laborious, difficult data analysis, hard to design primers, expensive
All *vs* All	HiC	Genome wide analysis	Relatively low resolution, very expensive, requires bioinformatic analysis
Many *vs* All	T2C	Superior (restriction fragment) resolution	Expensive , difficult data analysis requires advanced bioinformatics analysis
All *vs* All	Capture C	High resolution and high throughput in a single assay, identification of functional effects of SNPs	Difficult analysis, designing OCT oligos is challenging, unsuitable for *trans*-chromatin interactions

formation of a physical interaction between genes and their regulatory elements separated by large genomic distances (Hagege *et al*., 2007). The 3C technology is suited to study the three-dimensional chromatin structure in genomes, from bacteria to humans (Miele & Dekker, 2009). In addition to the detection of chromatin interactions within the same chromosome, the 3C assay has been also used to detect and study the *trans* interactions between regulatory elements located on different chromosomes (Miele & Dekker, 2009).

The first step in the 3C method is to establish the spatial organization of chromatin in living cells. For this purpose, chromatin is fixed with formaldehyde to crosslink the interacting fragments of DNA sequences and their regulatory elements. Crosslinked chromatin is then digested with a restriction enzyme. The most common ones, such as HindIII (Blackledge *et al*., 2009), BglII (Davison *et al*., 2012), BamHI (Hagege *et al*., 2007), EcoRI (Palstra *et al*., 2003) and DdeI (Salem *et al*., 2013), recognize 6 base pairs. At the following step, the sticky ends of the cross-linked DNA fragments are intramolecularly ligated by T4 DNA ligase. At this step, the cross-linked chromatin fragments, which are physically separated by a large distance in the genome, can be ligated to each other. This results in the formation of a three dimensional structure of chromatin in living cells. The crosslinks are then reversed and the DNA is purified. The final DNA template called "3C library" is analyzed to identify the frequency of interaction of the ligation products (Fig. 1). This analysis is performed via semi-quantitative PCR (Dekker *et al*., 2002) or quantitative real-time PCR using the Taqman probe and primers specific for the restriction fragments of interest (Splinter *et al*., 2006; Hagege *et al*., 2007). The mere detection of a ligation product between the two segments does not provide any information about the organization of chromatin. In order to identify the chromatin loop between the two segments of interest, a more frequent interaction between those DNA fragments than between the neighboring ones, has to be demonstrated. Therefore, the 3C analysis is based on the determination of accurate quantitative data on various ligation products using real-time PCR (Hagege *et al*., 2007).

The original 3C study was carried out on yeast chromosomes of *Saccharomyces cerevisiae* (Dekker *et al*., 2002). The 3C analysis detects the three-dimensional conformation of a chromosome in the nucleus and its changes during meiosis. The experiment identified the spatial architecture of yeast chromosome III, showing that it forms a contorted ring (Dekker *et al*., 2002). The following step was to use the 3C method to investigate the spatial organization of the beta-globin locus (*Hbb*) in mice (Tolhuis *et al*., 2002). It was found that a chromatin loop is formed in the region of the beta-globin locus that causes physical interaction between the locus control region (LCR) of beta-globin, located some distance upstream of the *Hbb* genes, and the active beta-globin locus. Subsequent studies showed that the formation of a chromatin loop in this region could potentially be the main mechanism of expression of the beta-globin locus (Tolhuis *et al*., 2002). The 3C analysis of the beta-globin locus in mouse erythroid cells showed changes in the three dimensional organization of the *Hbb* locus upon cell differentation (Palstra *et al*., 2003). In undifferentiated erythroid progenitor cells that do not express the *Hbb* gene, chromatin forms a chromatin hub (CH), but upon differentiation, in an actively transcribed gene, the chromatin hub is changed into an active chromatin hub (ACH). This confirms that the formation of loops between transcription factors and the target genes regulates the developmental expression of the beta-globin gene (Palstra *et al*., 2003). In contrast, in brain cells, where the globin genes are silent, the *Hbb* locus has a linear conformation which is consistent with the inactivation of the *Hbb* gene (McBride & Kleinjan, 2004).

USE OF CHROMOSOME CONFORMATION CAPTURE (3C) IN MEDICINE

Chromosome conformation capture became an interesting tool to determine the mechanism of tissue specific *CFTR* expression. The cystic fibrosis transmembrane conductance regulator (CFTR), when mutated, causes a common hereditary disorder, cystic fibrosis. *CFTR* expression is tissue-specific and is regulated temporally: it is expressed primarily in epithelial cells and is tightly regulated during development. The 3C analysis was used to examine the spatial organization of chromatin in the *CFTR* locus (Ott *et al*., 2009a; Ott *et al*., 2009b) and the fragments that flank the *CFTR* gene at -20.9 kbps and 15.6 kbps 5' and 3', respectively (Zhang *et al*., 2012). It was found that cells with high *CFTR* expression demonstrate strong interactions between intronic cis-acting regulatory elements and the *CFTR* promoter. In contrast,

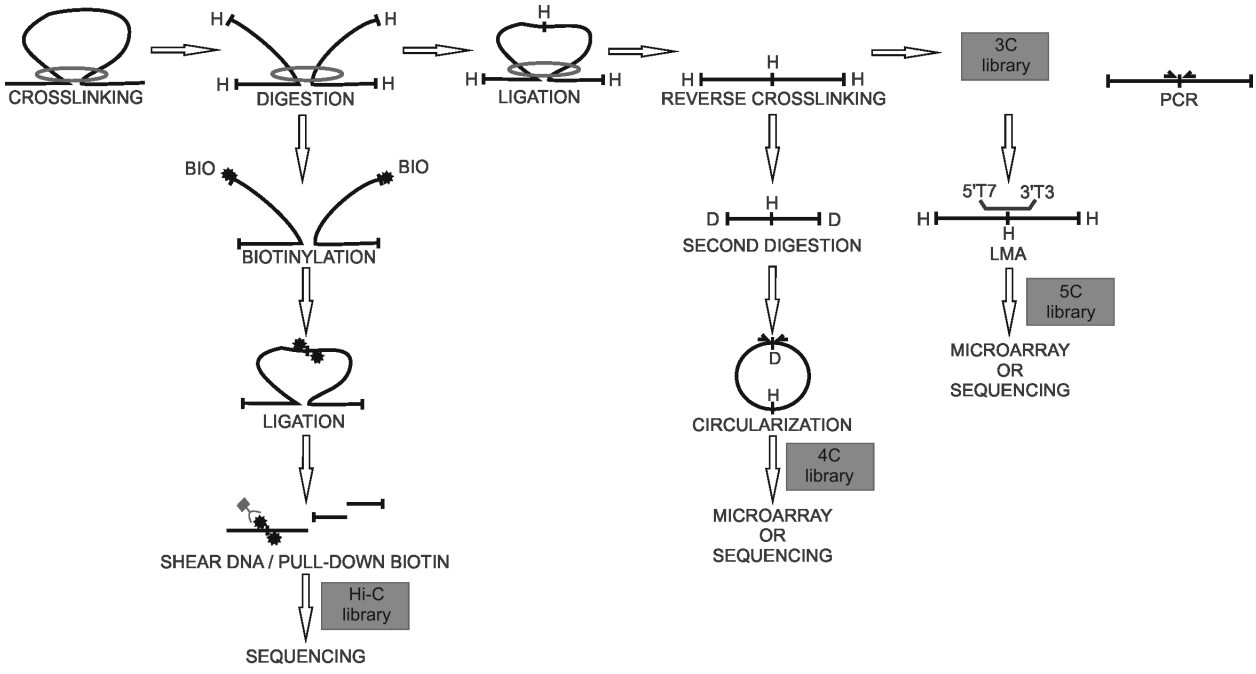

Figure 1. Overview of 3C and 3C-derived technologies.
The scheme presents the main stages of 3C, involving crosslinking of the interacting chromatin segments using formaldehyde, DNA digestion with an appropriate restriction enzyme and intramolecular ligation. The vertical deviation from the main figure shows the main steps specific to 3C-based methodologies, such as HiC (from digestion), 4C (from reverse crosslinking) and 5C (from the last step of 3C library preparation).

there are no significant interactions between the *CFTR* promoter and distal parts of the gene in skin fibroblasts. The chromatin loop between the *CFTR* promoter and the cis-regulatory elements located in intron 1 and at the 3' end of the gene is responsible for a tissue-specific mechanism of activation of the *CFTR* gene (Ott *et al.*, 2009a).

Another example how 3C analysis helps to understand the mechanisms of gene expression regulation was demonstrated in a gene associated with autoimmune diseases. The *TNFAIP3* locus encodes the ubiquitin-editing protein A20, which plays a central role in maintaining homeostasis of the immune system. Lower expression of A20 is responsible for the genetic association of systemic lupus erythematosus (SLE) (Wang *et al.*, 2013). Using chromatin conformation capture (3C), Wang and coworkers examined the mechanism of transcriptional regulation of the *TNFAIP3* gene through a long-range physical interaction (Wang *et al.*, 2013). The single nucleotide polymorphisms rs148314165 and rs200820567 reside in an enhancer element localized 42 kb downstream of the *TNFAIP3* promoter. It was shown that this enhancer allows binding of the NF-$\varkappa$B and SATB1 factors with the *TNFAIP3* promoter through long-range DNA looping (Wang *et al.*, 2013).

Another example of the use of the 3C assay in medical science is the analysis of chromatin organization in the genes involved in adipogenesis. It has been shown that a long range interaction occurs between the *PPARγ2* locus responsible for adipose differentiation and other adipogenic genes, such as adipokine or perilipin (LeBlanc *et al.*, 2014). Chromatin conformational changes are closely related to cell differentiation during adipogenesis and the formation of this intergenic interaction between the promoter of *PPARγ2* and other promoters associated with adipogenesis requires signals from C/EBPb, cAMP and protein kinase A. The presence of higher-order chromosomal structures probably serves as a label of tissue-specific gene expression for subsequent transcriptional activation during adipogenesis (LeBlanc *et al.*, 2014).

CHROMOSOME CONFORMATION CAPTURE 4C

The 3C analysis gave an opportunity to understand possible chromosomal interactions between the known 2 regions of interest using semi-quantitaive PCR, but did not permit screening of the whole genome for sequences that contact the regions of interest. Therefore, it was natural to include microarrays or new generation sequencing in a novel technology — 4C.

The 4C technology allows the evaluation of long-range chromatin interactions with large regions on the same chromosome (in *cis*) or on other chromosomes (in *trans*) using microarrays (Simonis *et al.*, 2006; Zhao *et al.*, 2006), or next-generation sequencing (NGS) (Splinter & de Laat, 2011).

CHROMOSOME CONFORMATION CAPTURE-ON-CHIP

The first steps in a 4C assay (Simonis *et al.*, 2006) are the same as described previously in the 3C method (Dekker *et al.*, 2002) and the differences start after DNA digestion. In the 4C method, a secondary digestion with additional restriction enzymes is performed, then DNA is purified and religated to form small DNA circles that are amplified by inverse PCR using bait-specific external primers. The PCR products represent the genomic environment of the fragments in contact at the chromosomal site of interest. Finally, the 4C template is labeled and hybridized to arrays according to standard chromatin immunoprecipitation (ChIP)-chip protocols (Fig. 1) (Si-

monis *et al*., 2006). A modification of chromosome conformation capture-on-chip is the 4C-seq method (Splinter & de Laat, 2011), which differs in technology. In the 4C-seq assay, the final 4C library is analyzed using next-generation sequencing (NGS) instead of microarrays.

CIRCULAR CHROMOSOME CONFORMATION CAPTURE

The principle of an alternative 4C method (Zhao *et al*., 2006) is the same as in the case of chromosome conformation capture-on-chip. The differences are in the protocol, in which the circular DNA step involves generation of circular DNA molecules under a high concentration of T4 DNA ligase and prolonged incubation (1 week). After this step, DNA is purified and amplified using nested PCR. The final 4C template is labeled and hybridized to microarrays.

The 4C technology was first used to investigate the chromosomal interaction profile of the mouse beta-globin locus (Simonis *et al*., 2006). It has been shown that the actively transcribed β-globin gene in the fetal liver has a completely different profile of chromatin interactions on chromosome 7 from the transcriptionally silent β-globin locus in the brain tissue. These studies demonstrated a completely different architecture of chromatin of the mouse β-globin gene, depending on its expression status (Simonis *et al*., 2006).

USE OF CHROMOSOME CONFORMATION CAPTURE (4C) IN MEDICINE

The 4C strategy is known as a "one *versus* all" because the genome is screened for sequences that contact the selected site (Fullwood *et al*., 2009). Therefore, it is more likely that more clinical uses can be found by this 4C technology or its successors.

In fact, 4C-seq was used to analyze the chromain interaction profiles in breast cancer cell lines (Zeitz *et al*., 2013). It has been shown that the *IGFBP3* gene, associated with the pathogenesis of breast cancer, has a different chromatin architecture in breast cancer cells compared to normal cells. Furthermore, a significant chromosomal interaction between the *EGFR* and *IGFBP3* genes was found, and this interaction potentially may play a role in their regulation, and the results have to be checked and confirmed in functional applications.These data suggest a possible role of long-range chromatin interactions in the pathogenesis of breast cancer (Zeitz *et al*., 2013).

Another chromosome conformation capture-on-chip (4C) assay was performed to examine the spatial organization of the *SOX9* locus and to identify potentially novel regulatory elements that might be responsible for sex reversal in patients with campomelic dysplasia (CD) (Smyk *et al*., 2013). Smyk *et al*. identified several novel potentially cis-interacting regions, both up- and downstream of the promoter of the gene, that affect the regulation of the expression of *SOX9*.

CHROMOSOME CONFORMATION CAPTURE 5C

Chromosome conformation capture carbon copy (Dostie *et al*., 2006) allows a parallel analysis of interactions between many selected loci. The 5C method is used to map large-scale inter- and intrachromosomal contacts between genomic elements, and to study higher order chromosome structure. The 5C method can be also used for designing the complete map of chromatin looping interactions for each genomic region of interest (Dostie *et al*., 2006).

The 5C assay, as one of the 3C-derived methods, detects ligation products in 3C libraries by a highly multiplexed ligation-mediated amplification (LMA). The 3C template is hybridized to a mix of oligonucleotides (oligo-5C), which overlaps specific restriction sites in the tested sequences and can be ligated together. This ligation-mediated amplification step generates the 5C library. Due to the presence of unique sequences in oligo-5C primers, all ligation products can be amplified in a multiplex PCR reaction. All ligation products are analyzed using microarrays or high-throughput sequencing (Fig. 1) (Dostie *et al*., 2006).

The first studies employing the 5C technology involved the analysis of chromatin structure in the human beta-globin (Dostie *et al*., 2006) and alfa-globin (Bau *et al*., 2011) genes. The chromatin interaction profile of the beta-globin locus was previously identified *via* the 3C assay (Tolhuis *et al*., 2002), so the beta globin gene was used for the validation of the 5C method. Dostie and coworkers (2006) identified several known chromatin loops in the beta-globin locus which confirms the effectiveness of the 5C method. The 5C analysis also indicated novel looping interactions between the beta-globin Locus Control Region (LCR) and the gamma-beta intergenic region, and these results showed that the 5C method is more accurate and more efficient than the traditional 3C analysis (Dostie *et al*., 2006).

USE OF CHROMOSOME CONFORMATION CAPTURE (5C) IN MEDICINE

One of the examples of the use of 5C in medical sciences is the spatial organization analysis of chromatin in the *HOX* locus (Wang *et al*., 2011). High throughput chromosome conformation capture across the *HOX* gene revealed that its chromatin interaction profile is dependent on anatomical identity. There are significant differences in the higher order chromatin structure of the *HOX* genes, depending on their localization in distal cells (fibroblasts) or proximal cells (lung epithelium). Different interaction profiles also depend on the transcriptional activity of the *HOX* gene. In fibroblasts, chromatin loop occurs in the 5' *HOX* locus, where the locus is transcriptionally active, but there are no long-range chromatin interactions within the transcriptionally silent 3' *HOX* (Wang *et al*., 2011).

Another use of the 5C assay was to explore whether chromatin conformation can be used to classify human leukemia (Rousseau *et al*., 2014). The 5C assay was used to measure chromatin contacts throughout the *HOXA* cluster region in a panel of leukemia cell lines that featured lymphoblastic (ALL) and myeloid (AML) leukemia. The results showed that the *HOXA* chromatin interaction profiles in leukemia samples expressing MLL fusions are sufficiently different from those encoding wild-type MLL. This study demonstrated that chromatin interactions in the *HOXA* gene yield valuable information necessary for the classification of leukemia subtypes (Rousseau *et al*., 2014).

HIGH-RESOLUTION CHROMOSOME CONFOMATION CAPTURE (Hi-C)

Last but not least, the Hi-C technology (Lieberman-Aiden *et al*., 2009) allows studying the three-dimensional architecture of chromatin in the whole genome using massively parallel sequencing techniques.

In Hi-C, the procedure of generating a 3C library is slightly different. The initial steps, chromatin crosslinking and DNA digestion with a restriction enzyme, are identical to the 3C method. Near the 5' restriction ends, biotin-labeled nucleotides are added and DNA is ligated under diluted conditions. The resulting DNA template contains products that originally were in close physical distance and ligation junctions are marked with biotin. The final Hi-C library is generated by shearing DNA and selecting the biotinylated fragments with streptavidin beads. The library is then analyzed using massively parallel DNA sequencing, producing matrix of interaction frequencies between all fragments in the genome (Fig. 1) (Lieberman-Aiden *et al*., 2009).

Pioneer studies using the Hi-C methodology resulted in the construction of spatial proximity maps of the human genome at the resolution of 1 Mbp (Dostie *et al*., 2006). These maps confirm the spatial organization of chromatin previously examined using fluorescence *in situ* hybridization (FISH) (Yokota *et al*., 1995) and chromosome conformation capture (3C) (Dekker *et al*., 2002).

Use of high-resolution chromosome confomation capture in medicine

The Hi-C method was used in medical sciences to create spatial interaction maps of the three-dimensional chromosome conformation with the collections of 1 533 chromosomal translocations from cancer and germline genomes (Engreitz *et al*., 2012). The authors demonstrated that genomic regions with translocation patterns observed in human diseases have higher Hi-C contact frequencies. This observation suggests that many translocation partners are located in the chromatin domains occurring in close proximity in normal cells, thus predisposing them to chromosomal rearrangements (Engreitz *et al*., 2012). Additionally, tissue-specific patterns of chromatin interactions in the human genome were evaluated and it was found that translocation breakpoints reported in human hematologic malignancies have higher Hi-C contact frequencies in lymphoid cells than those reported in other tumors. These results suggest that tissue-specific changes in the genome organization may predispose tissues to transformation into different malignancies (Engreitz *et al*., 2012).

TARGETED CHROMATIN CAPTURE T2C

T2C technology (Kolovos *et al*., 2014) allows the identification of chromatin interactions for specific genomic regions in *cis* and *trans* conformation. This high throughput technology can analyze genome compartmentalization and indicates regulatory elements at a superior resolution using HiSeq squencing.

The principle of T2C is the same as in the 3C method. The chromatin is crosslinked, DNA is digested with a restriction enzyme and intramolecularly ligated under diluted conditions. In the next step, the DNA undergoes a secondary digest with another restriction enzyme or is mechanically sheared to obtain small fragments containing the ligation site. Finally, the library is ligated with adapters followed by hybridization to a set of unique oligonucleotides on an array. The hybridized DNA, which contains the set of ligation products, is eluted and analyzed using Illumina-sequencing.

The first studies employing the Targeted Chromatin Capture T2C methodology involved analysis of chromatin interactions in the *H19/IGF2* region on human chromosome 11 (Kolovos *et al*., 2014). This well-known model region of chromatin conformation was chosen to validate T2C in comparison to 3C and 4C-seq technology. Generally, T2C methods indicate a similar interaction profile as observed with different chromatin conformation capturing methods but at a higher resolution (single restriction fragments). Next, Kolovos *et al*. used T2C to explore chromatin organization of the mouse beta-globin locus in terms of the transcriptional activity of the gene. It has been shown that the actively transcribed beta-globin loci in mouse primary erythroid cells from fetal liver has a higher interaction frequency than in fetal brain cells, when this locus is inactivated. In addition, for the β-globin promoter, a novel, additional chromatin interaction located approximately 1 Mb away from the promoter in erythroid cells was identified due to absence of this interaction in brain cells. Furthermore, T2C analysis explored a high chromatin interaction of the important chromatin proteins, Ldb1 and CTCF in actively transcribed beta-globin locus (Kolovos *et al*., 2014).

Taken together, all results demonstrate that T2C high throughput technology is an important tool to identify spatial chromatin organization and the compartmentalization of specific regions of the genome at high single restriction fragment resolution, for both clinical and non-clinical research.

CAPTURE C

Capture C (Hughes *et al*., 2013) is a high throuhgput technology that allows to analyze long-range chromatin interactions in cis conformation at hundreds of selected loci at high resolution in a single assay. Capture C uses 3C, oligonucleotide capture technology (OCT) and high-throughput sequencing for mapping chromatin cis interaction profile at a genome-wide scale. Moreover, capture C is a useful tool to link regulatory SNPs to the genes whose expression they control.

The principles of Capture C are the same as in conventional 3C technology. Chromatin is fixed, followed by digestion and intramolecular ligation. In addition, in Capture C, 3C template is sonicated, and sequencing adaptors are attached. Finally, 3C library is ligated with specific oligonucleotides spanning 5' and 3' restriction sites of interest by an OCT protocol. The loci of interest and their interaction partners with captured oligos are amplified and sequenced by Illumina-sequencing (Hughes *et al*., 2013).

Capture C was validated by analysis of alpha- and beta-globin genes — two regions of the genome used as the reference genes in chromatin conformation capture technology. Capture-C reproducibly identified all previously described *cis* interactions within the globin loci and also all of these interaction were analyzed in a single experiment (Hughes *et al*., 2013).

Another analysis using capture C was to link SNPs in regulatory elements to genes that they affect. The aim was to determine the effect of distal SNP on gene regulation in mouse eryhroid cells. Hughes and coworkers (2013) link SNPs found in regulatory elements to the genes whose expression they influence. For example, they discovered functional effects of SNPs that bind transcription factor GATA1 and Sc1 to an enhancer that linked the promoter of the *PNPO* gene and highly increased expression of the *PNPO* gene in erythriod cells (Hughes *et al*., 2013).

CONCLUDING REMARKS

The spatial architecture of chromatin plays an important role in the mechanism of gene expression in the eukaryotic genome (Wolffe & Hayes, 1999; Dietzel *et al*., 2004; Rodriguez & Bjerling, 2013). In this article, we present different methods of exploration of the three dimensional organization of chromatin in living cells. Starting from 3C, the technology that indicated chromatin loops formed in genomic regions of up to several hundred kilobases, through the 3C-based methods 4C, 5C, Hi-C, Capture C and T2C that employ high-throughput technologies to analyze long range chromosome interactions in larger genomic regions. Chromosome conformation capture methods are powerful tools to model nuclear organization in detail. Knowledge of the three dimensional organization of nucleus has a direct impact on new transcription factor's and insulator's discovery as well as mechanisms of gene regulation in living cells. This will aid in understanding of the molecular mechanisms controlling chromatin organization, and the role of genome organization in regulating gene expression and other nuclear functions (Graaf & Steensel, 2013). 3C and 3C-based (4C, 5C, Hi-C, Capture C, T2C) studies demonstrate that gene expression patterns are associated with specific chromatin structures and may therefore correlate with chromatin conformation signatures (Cruthley *et al*., 2010).

Moreover, we focused on different aspects of using chromosome conformation technology in translational medicine and their clinical application. Chromatin conformation analysis made it possible to investigate the mechanism of gene expression regulation of genes involved in the pathogenesis of many human diseases and identification of many new transcription factors. Chromatin conformation technologies also indicate an important role of chromatin organization in tumor pathogenesis. The identification of chromatin interactions affecting the formation of chromosomal rearrangements within the human genome can give us important information related to their impact on the development of many diseases.

Furthermore, knowledge about three dimensional chromation organization of nucleus that play an important role in gene expression regulation of genes involved in cancerogenesis and many human diseases may apply in clinical therapies. Epigenetic modifications like histone modifications and chromatin remodeling are reversible and can be potentially targeted by series of epigenetic and histonomic drugs (Pecorino, 2013; Cacabelos, 2014). In the future, Chromatin Conformation technologies may be applied in pharmacoepigenetic studies by generation of new drugs and therapies that would affect epigenetic modulation of chromatin via chemical structural interactions with epigenetic enzymes, through interactions with DNA repair mechanisms (Kelly *et al*., 2010). Futhermore, chromosome conformation technologies may help to identify factors invloved in forming chromatin loops for e.g. CTCF or STAB1. The identification of factors called chromatin conformation signatures (CCSs) may represent novel biomarkers of human disease and help to identify novel pathological mechanisms (Cruthley *et al*., 2010). These clinical implications of chromatin conformation technologies have an important impact on personalized medicine.

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REFERENCES

Bau D, Sanyal A, Lajoie BR, Capriotti E, Byron M, Lawrence JB, Dekker J, Marti-Renom MA (2011) The three-dimensional folding of the alpha-globin gene domain reveals formation of chromatin globules. *Nat Struct Mol Biol* **18**: 107–114.

Blackledge NP, Ott CJ, Gillen AE, Harris A (2009) An insulator element 3' to the CFTR gene binds CTCF and reveals an active chromatin hub in primary cells. *Nucleic Acids Res* **37**: 1086–1094.

Cacabelos R (2014) Epigenomic Networking in drug development: from pathogenic mechanisms to pharmacogenomics. *Drug Develop Res* **75**: 348–365.

Carter D, Chakalova L, Osborne CS, Dai YF, Fraser P (2002) Long-range chromatin regulatory interactions *in vivo*. *Nature Genet* **32**: 623–626.

Crutchley JL, Wang XQ, Ferraiuolo MA, Dostie J (2010) Chromatin conformation signatures: ideal human disease biomarkers? *Biomark Med* **4**: 611–629.

Davison LJ, Wallace C, Cooper JD, Cope NF, Wilson NK, Smyth DJ, Howson JMM, Saleh N, Al-Jeffery A, Angus KL, Stevens HE, Nutland S, Duley S, Coulson RMR, Walker NM, Burren OS, Rice CM, Cambien F, Zeller T, Munzel T, Lackner K, Blankenberg S, The Cardiogenics Consortium, Fraser P, Gottgens B, Todd JA (2012) Long-range DNA looping and gene expression analyses identify DEXI as an autoimmune disease candidate gene. *Hum Mol Genet* **21**: 322–333.

Dekker J, Rippe K, Dekker M, Kleckner N (2002) Capturing chromosome conformation. *Science* **295**: 1306–1311.

Dietzel S, Zolghadr K, Hepperger C, Belmont AS (2004) Differential large-scale chromatin compaction and intranuclear positioning of transcribed *versus* non-transcribed transgene arrays containing beta-globin regulatory sequences. *J Cell Sci* **117**: 4603–4614.

Dostie J, Richmond TA, Arnaout RA, Selzer RR, Lee WL, Honan TA, Rubio ED, Krumm A, Lamb J, Nusbaum C, Green RD, Dekker J (2006) Chromosome Conformation Capture Carbon Copy (5C): a massively parallel solution for mapping interactions between genomic elements. *Genome Res* **16**: 1299–1309.

Engreitz JM, Agarwala V, Mirny LA (2012) Three-dimensional genome architecture influences partner selection for chromosomal translocations in human disease. *PloS One* **7**: e44196.

Fullwood MJ, Liu MH, Pan YF, Liu J, Xu H, Mohamed YB, Orlov YL, Velkov S, Ho A, Mei PH, Chew EG, Huang PY, Welboren WJ, Han Y, Ooi HS, Ariyaratne PN, Vega VB, Luo Y, Tan PY, Choy PY, Wansa KD, Zhao B, Lim KS, Leow SC, Yow JS, Joseph R, Li H, Desai KV, Thomsen JS, Lee YK, Karuturi RK, Herve T, Bourque G, Stunnenberg HG, Ruan X, Cacheux-Rataboul V, Sung WK, Liu ET, Wei CL, Cheung E, Ruan Y (2009) An oestrogen-receptor-alpha-bound human chromatin interactome. *Nature* **462**: 58–64.

De Graaf CA, van Steensel B (2013) Chromatin organization: form to function. *Curr Opin Genet Dev* **23**: 185–190.

Hagege H, Klous P, Braem C, Splinter E, Dekker J, Cathala G, de Laat W, Forne T (2007) Quantitative analysis of chromosome conformation capture assays (3C-qPCR). *Nature Protocols* **2**: 1722–1733.

Hughes JR, Roberts N, McGowan S, Hay D, Giannoulatou E, Lynch M, De Gobbi M, Taylor S, Gibbons R, Higgs DR (2014) Analysis of hundreds of cis-regulatory landscapes at high resolution in a single, high-throughput experiment. *Nature Genet* **46**: 205–212.

Kelly TK, De Carvalho DD, Jones PA (2010) Epigenetic modifications as therapeutic targets. *Nature Biotechnol* **28**: 1069–1078.

Kolovos P, van de Werken HJ, Kepper N, Zuin J, Brouwer RW, Kockx CE, Wendt KS, van IJcken WF, Grosveld F, Knoch TA (2014) Targeted Chromatin Capture (T2C): a novel high resolution high throughput method to detect genomic interactions and regulatory elements. *Epigenetics & Chromatin* **7**: 10.

LeBlanc SE, Wu Q, Barutcu AR, Xiao H, Ohkawa Y, Imbalzano AN (2014) The PPARgamma locus makes long-range chromatin interactions with selected tissue-specific gene loci during adipocyte differentiation in a protein kinase A dependent manner. *PloS One* **9**: e86140.

Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, Sand-

strom R, Bernstein B, Bender MA, Groudine M, Gnirke A, Stamatoyannopoulos J, Mirny LA, Lander ES, Dekker J (2009) Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* **326**: 289–2893.

McBride DJ, Kleinjan DA (2004) Rounding up active cis-elements in the triple C corral: combining conservation, cleavage and conformation capture for the analysis of regulatory gene domains. *Briefings Funct Genom Proteom* **3**: 267–279.

Miele A, Dekker J (2009) Mapping cis- and trans- chromatin interaction networks using chromosome conformation capture (3C). *Methods Mol Biol* **464**: 105–121.

Ott CJ, Blackledge NP, Kerschner JL, Leir SH, Crawford GE, Cotton CU, Harris A (2009a) Intronic enhancers coordinate epithelial-specific looping of the active CFTR locus. *Proc Natl Acad Sci USA* **106**: 19934–19939.

Ott CJ, Blackledge NP, Leir SH, Harris A (2009b) Novel regulatory mechanisms for the *CFTR* gene. *Biochem Soc Trans* **37**: 843–848.

Palstra RJ, Tolhuis B, Splinter E, Nijmeijer R, Grosveld F, de Laat W (2003) The beta-globin nuclear compartment in development and erythroid differentiation. *Nature Genet* **35**: 190–194.

Pecorino L (2012) Epigenomic and histonomic drugs. In *Molecular Biology of cancer Mechnisms, Targets and Therapeutics*, pp 70–72. Oxford University Press, Oxford.

Rodriguez A, Bjerling P (2013) The links between chromatin spatial organization and biological function. *Biochem Soc Trans* **41**: 1634–1639.

Rousseau M, Ferraiuolo MA, Crutchley JL, Wang XQ, Miura H, Blanchette M, Dostie J (2014) Classifying leukemia types with chromatin conformation data. *Genome Biol* **15**: R60.

Salem T, Gomard T, Court F, Moquet-Torcy G, Brockly F, Forne T, Piechaczyk M (2013) Chromatin loop organization of the junb locus in mouse dendritic cells. *Nucleic Acids Res* **41**: 8908–8925.

Sexton T, Bantignies F, Cavalli G (2009) Genomic interactions: Chromatin loops and gene meeting points in transcriptional regulation. *Semin Cell Dev Biol* **20**: 849–855.

Simonis M, Klous P, Splinter E, Moshkin Y, Willemsen R, de Wit E, van Steensel B, de Laat Wet (2006) Nuclear organization of active and inactive chromatin domains uncovered by chromosome conformation capture-on-chip (4C). *Nature Genet* **38**: 1348–1354.

Smyk M, Szafranski P, Startek M, Gambin A, Stankiewicz P (2013) Chromosome conformation capture-on-chip analysis of long-range cis-interactions of the SOX9 promoter. *Chromosome research: an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology*. **21**: 781–788.

Splinter E, de Laat W (2011) The complex transcription regulatory landscape of our genome: control in three dimensions. *EMBO J* **30**: 4345–4355.

Splinter E, Heath H, Kooren J, Palstra RJ, Klous P, Grosveld F, Galjart N, de Laat W (2006) CTCF mediates long-range chromatin looping and local histone modification in the beta-globin locus. *Genes Develop* **20**: 2349–2354.

Tolhuis B, Palstra RJ, Splinter E, Grosveld F, de Laat W (2002) Looping and interaction between hypersensitive sites in the active beta-globin locus. *Mol Cell* **10**: 1453–1465.

Wang KC, Yang YW, Liu B, Sanyal A, Corces-Zimmerman R, Chen Y, Lajoie BR, Protacio A, Flynn RA, Gupta RA, Wysocka J, Lei M, Dekker J, Helms JA, Chang HY (2011) A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. *Nature* **472**: 120–124.

Wang S, Wen F, Wiley GB, Kinter MT, Gaffney PM (2013) An enhancer element harboring variants associated with systemic lupus erythematosus engages the TNFAIP3 promoter to influence A20 expression. *PLoS Genetics* **9**: e1003750.

Wolffe AP, Hayes JJ (1999) Chromatin disruption and modification. *Nucleic Acids Res* **27**: 711–720.

Yokota H, van den Engh G, Hearst JE, Sachs RK, Trask BJ (1995) Evidence for the organization of chromatin in megabase pair-sized loops arranged along a random walk path in the human G0/G1 interphase nucleus. *J Cell Biol* **130**: 1239–1249.

Zeitz MJ, Ay F, Heidmann JD, Lerner PL, Noble WS, Steelman BN, Hoffman AR (2013) Genomic interaction profiles in breast cancer reveal altered chromatin architecture. *PloS One* **8**: e73974.

Zhang Z, Ott CJ, Lewandowska MA, Leir SH, Harris A (2012) Molecular mechanisms controlling CFTR gene expression in the airway. *J Cell Mol Med* **16**: 1321–3130.

Zhao Z, Tavoosidana G, Sjolinder M, Gondor A, Mariano P, Wang S Kanduri C, Lezcano M, Sandhu KS, Singh U, Pant V, Tiwari V, Kurukuti S, Ohlsson R (2006) Circular chromosome conformation capture (4C) uncovers extensive networks of epigenetically regulated intra- and interchromosomal interactions. *Nature Genet* **38**: 1341–1347.