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Review Article

The Future of 3D Genomic Technology: Precision-Omics

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ABSTRACT

Three-dimensional (3D) genomic technologies are widely used in recent years. These technologies, mainly C-technologies, burst the research regarding the relationships between 3D chromatin conformation and gene transcriptional regulation. Although these methods help the understanding of chromatin biology with a new perspective, the 3D nucleome information is still imprecise. So far, as the limitations, the C-technology data are always high-noise with poor resolution. Besides, they redefine the widely known 3D genome structures and reveal dynamic chromatin conformation change. However, the redefinition and reveal from current technologies are mostly in population-average cell levels. In this review, based on our research and reported literatures, we briefly discuss the current efforts and the future of 3D genomic technologies in population-cell level, single-cell level and single-molecule level.

Keywords: 3D genomic technology; Chromatin conformation; Hi-C

INTRODUCTION

Genome carries the genetic information of organisms. The patterns of genome elegantly folding in nucleus and precisely guiding life activities are fascinating. Traditionally, scientists have preferred using microscopical techniques to decipher these phenomena, from chromatin 3D structures to gene functions. But the limitations of resolution and throughput restrain scientists observing the appropriate scale gene level interactions (promoter-enhancer interaction, promoter-silencer interaction. etc) and genome-wide 3D chromatin landscapes [1].

In recent years, with the advance of biochemistry and molecular biology technologies, scientists develop chromosome conformation capture technologies (C-technologies) which not only can be used to reveal gene-gene, gene-intergenic region interactions, but also reconstruct genome-wide chromatin 3D conformations. C-technologies include 3C, 4C, 5C, Hi-C and their derivatives. With these technologies, different 3D genomic architectures are redefined, such as chromatin territory, A/B compartment, topologically associating domains (TADs), sub-TADs and DNA-loops [2]. These further the knowledge revealed by microscopy techniques. Some structures can be cross-validated.

Although C-technologies are leading the trend of 3D genomics research now and might be in the future, there are still some questions need to be solved. The data or results of existing C-technologies are high-noise, these should be improved from the NGS C-library construction. High quality and reliable data is the premise to correctly illuminate the precise 3D structures of chromatin [2]. And that, these widely used bulk C-technologies capture the 3D interactions and conformations in population cell level. These would lose the individual feature of single cell, because sometimes cell population owns the feature of heterogeneity [3]. Efficient single cell C-technology, e.g. single cell-Hi-C, is still needed to shed light on the life activities of cells, tissues or organisms comprehensively [4,5]. Furthermore, these pairwise proximity-ligation based C-technologies are also not comprehensive in capturing interactions, because the chromatin interactions mediated by a complex are always multiplex. Capturing the whole multiplex interactions in single-molecule complex would support abundant interaction information, even under the situation cells are rarely.

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Capturing three-dimensional genome structures by noise reduced population-cell C-technology

In the last two decades, C-technologies impel the genomics research from one dimension (1D) to 3D [6]. These methods help scientists discover a lot of enchanting biological phenomena, involving chromatin conformation, transcriptional regulation, cell division and death, cell growth, differentiation and genome evolution [7]. These methodologies are mainly based on proximity-ligation and derived from traditional 3C technology. But there are generally high noise and low resolution. Kong et al. proposes a strategy to reduce noises in 3D genomic technologies [8].

Under C-technology proximity-ligation circumstance, there would be miscellaneous undesired linear DNAs, i.e. main noise sources, needed to be eliminated [2]. In their research, adopting plasmid test, different exonuclease combinations were pre-tested to remove linear DNA. They found that LRL (Lambda and RecJF within Lambda buffer), LRC (Lambda and RecJF within Cutsmart buffer), LIC (Lambda and Exonuclease I within Cutsmart buffer) and IIIIC (Exonuclease I and Exonuclease III within Cutsmart buffer) combinations are working and can eliminate the linearized DNA in the appropriate conditions. As Hi-C is the flagship C-technology, in this study, linear DNA eliminating step (exo-) was introduced after Hi-C proximity-ligation. They were conducted on the traditional standard Hi-C (Exo-Hi-C) [9] and *in situ* Hi-C (*in situ* exo-Hi-C) [10], respectively. For exo-Hi-C, the valid interaction pairs ratio increases from ~40% to ~80%. 3D genome characteristics are robust after noise elimination, such as defined A/B compartment, domains, gene clusters and promoter-enhancer loops. The application in *in situ* Hi-C (*in situ* exo-Hi-C) showed that the number of DNA-Loops is greatly increased, which has a potential to improve the accuracy of gene function interpretation. Theoretically, this strategy would be applied to a wide range of C-technologies (Figure 1) [8].

Thus, this study supports one of the methods to reducing noises and efficiently capturing the interactions. Precisely capturing valid interactions can support more accurate data used for reconstructing 3D chromatin conformation and acquiring exact interaction information between functional genes and transcription regulation elements. Therefore, more efficient and precise 3D genomic technologies are needed in the future.

Casting light on the cell heterogeneity of chromatin conformation: from bulk to single cell

Since the conformation information revealed by the widely used Hi-C is always the population average level, we are unable to define what the real status of each cell in this population is [1,11]. And often the results of population levels are likely to be different from those of individual cells. Interestingly, the single-cell Hi-C technology that can be performed on single cell, which could be used to accurately study the single-cell individual genomic interaction patterns, local domain characteristics, global conformation and other information about cellular heterogeneity [12]. Single-cell Hi-C would be another future development trend of precise 3D genomic technology.

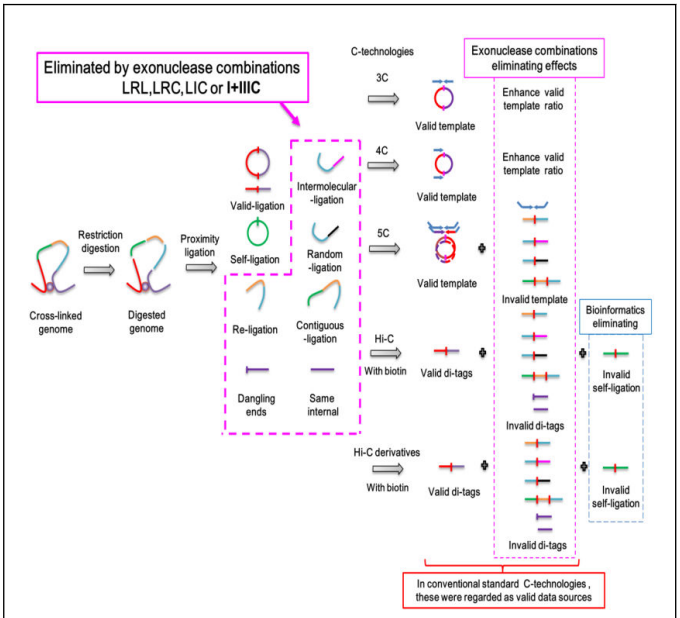

Figure 1: Exonuclease combination reducing noises is a good strategy for improving 3D genomic methodology [8].

In Figure 2, we mainly summarize the current technological progress of single-cell Hi-C technologies, including Nagano 2013 Single-cell Hi-C [11], Nagano 2017 Single-cell Hi-C [13], snHi-C [14], Stevens 2017 single-cell Hi-C [15], Single-cell combinatorial indexed Hi-C (sciHi-C) [16] and Dip-C [12]. However, single-cell Hi-C is far lower than bulk Hi-C in valid data (removing duplication) (20 K-1.9 M valid pairs), coverage and resolution (~100 kb) [17]. Currently, single-cell Hi-C technique still requires high skill operation. Operating on a single cell in a single well is low through-put and time-consuming. It is not easy to keep stable because output data is quite different, which is difficult to obtain relatively consistent data and results like bulk Hi-C. And in these single-cell Hi-C technologies, a single cell DNA needs 25-cycle PCR to yield enough DNA, which results in influencing the ratio of valid data and often produces abnormal fragments.

By adding unique label to the single cell in the population cells, and then mixing the labelled cells to operate, this can help to achieve high efficiency and high throughput. High-fidelity genome-wide amplification prior to PCR can also increase the amount of DNA templates. Both are important improved directions of single-cell Hi-C need attention in the future.

Single-molecule 3D genome capturing technology would provide more precise multiplex interaction information

Although high-throughput sequencing expands the detection range to the whole genome level and chromatin immunoprecipitation makes it possible to detect the interaction mediated by specific proteins, the genome-wide interaction matrix obtained by C-technology will emerge sparse trend when the input cell numbers are decreased to very low, especially single cell [11]. The reason may be that the widely used C-technologies are always based on pairwise proximity-ligation [2]. But in the single-molecule interaction complex, there may not

be just two interactive DNA fragments. Three or more fragments could be the real status [18]. Therefore, capturing the comprehensive interactions in each single-molecule interaction complex in cells would be a valid solution.

Interestingly, Zheng et al. creatively proposes a multiplex chromatin interaction capture method called ChIA-Drop [18]. ChIA-Drop separates single-molecule chromatin interaction complex into unique drop which helps to realize barcode-linked amplification. DNA-fragments with the same barcode are considered to be interactive. As this method overcomes traditional pairwise ligation drawbacks and brings many novel biological phenomena. Some of them are different from the former results observed by pairwise ligation C-technologies. ChIA-Drop found chromatin interaction complex comprises various interactions with high heterogeneity (form two to hundreds of interactions). Some complexes with large numbers of fragments are clustered and similar to TADs defined by Hi-C. Some complexes with low numbers of DNA fragments are scattered and exist in TAD gap regions.

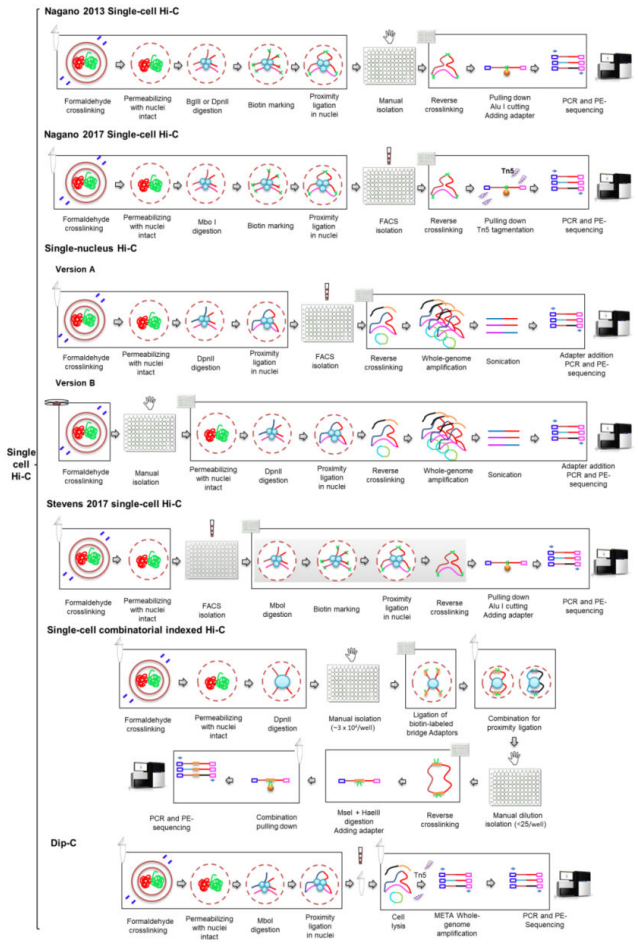

Figure 2: Single-cell Hi-C methodologies.

Comparing ChIA-Drop, RNAPII ChIA-Drop with RNAPII ChIA-PET [19], scientists found that in TAD gap regions (called RNAPII associated interaction domains, RAIDs), the contacts defined by RNAPII ChIA-Drop and RNAPII ChIA-PET are abundant and overlapped [18]. But RNAPII ChIA-Drop and RNAPII ChIA-PET reflect few signals in TAD regions, which are different from ChIA-Drop and Hi-C signal situations [20]. These would be some of the reasons to TAD controversy [2,21].

Here, for ChIA-Drop, TADs are newly regarded as inhibitory transcription regions. And the boundary gaps of TADs are transcriptionally active. These are different from the previous view that TADs are transcriptional regulation microenvironment [2,22]. In addition, different form before, 80% RNAPII mediated complexes have only one promoter. Two or more promoters in one complex are also existed and they are co-transcriptionally regulated, which is consistent with transcription factories. But most promoters genome-wide are not interconnected and in their unique complexes. Besides, RNAPII ChIA-Drop reveals an active promoter-centered multivalent interaction change during transcription. The transcriptional complex is like a processive reel, DNA template mediated by the complex from two fragments to several fragments (Figure 3). However, these would always be averaged when captured by pairwise traditional chromatin interaction analysis [18].

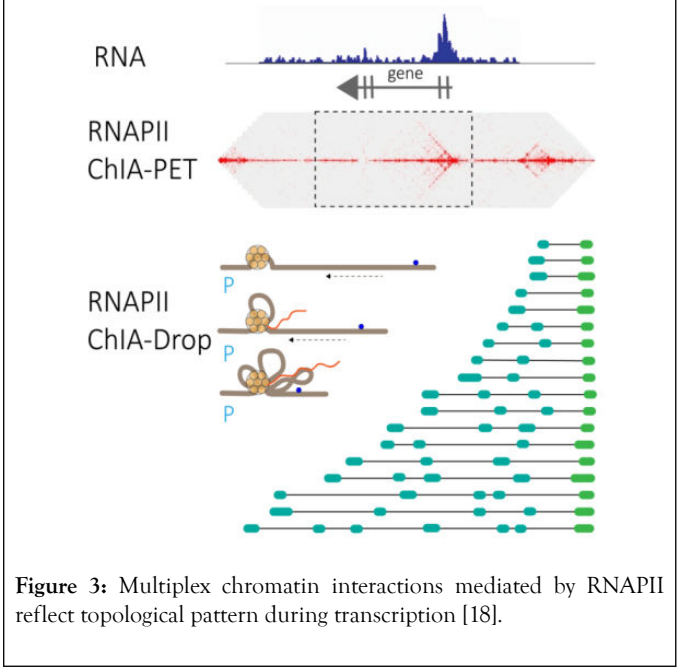

Figure 3: Multiplex chromatin interactions mediated by RNAPII reflect topological pattern during transcription [18].

For ChIA-Drop, it should be considered that there are several developing directions. First, the reference genome size of *Drosophila* S2 cells is small [19]. The consistency of result between big-size genome like human/mice and *Drosophila* S2 is unknown. Second, comparing with *in situ* Hi-C, the reproducibility of ChIA-Drop needs be optimized [10]. Third, the smaller DNA fragments are, the lower uniquely mappable read ratio is. This phenomenon makes obtaining smaller multiplex interaction complexes limited, restricts the resolution and valid data ratio [18]. The alignment algorithm might be needed to improve. Forth, when ChIA-Drop focuses on intra-chromosomal domain-domain long-range interactions, the signal density is higher than Hi-C. There may be a case that some nearby single-molecule multiplex interactive complexes (domains) are not interconnected but distributed into same droplet, which would be hard to be distinguished from the real

inter-domain contacts. Fifth, ChIA-Drop realized multiplex chromatin interaction capturing on single-molecule level, but it is still basing on population-cell level. As reported, all of the now available single-cell Hi-C technologies are under pairwise ligation. Here we take a wild guess. Multiplex chromatin interaction capture will provide an efficient approach to solve single-cell Hi-C library complexity and matrix sparsity limitation.

CONCLUSION

Capturing precise 3D genome structures can help to accurately elucidate biology function. More efficient and precise 3D genomic technologies are anticipated. In this review, we highlight three important directions: Noise elimination in population-cell level, high-throughput chromatin conformation capture in single cell and multiplex interaction barcoding in single molecule. They are gradually progressive and complementary to each other.

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AUTHOR INFORMATION

Siyuan Kong and Qitong Huang are co-first authors.

REFERENCES

1. Bintu B, Mateo LJ, Su J, Sinnottarmstrong NA, Parker M, Kinrot S, et al. Super-resolution chromatin tracing reveals domains and cooperative interactions in single cells. Science. 2018;362(6413):e1783.
2. Kong S, Zhang Y. Deciphering Hi-C: from 3D genome to function. Cell Biol Toxicol. 2019;35(1):15-32.
3. Ren X, Kang B, Zhang Z. Understanding tumor ecosystems by single-cell sequencing: promises and limitations. Genome Biol. 2018;19(1):211.
4. Monahan K, Horta A, Lomvardas S. LHX2- and LDB1-mediated trans interactions regulate olfactory receptor choice. Nature. 2019;565(7740):448-453.
5. Tan L, Xing D, Daley N, Xie XS. Three-dimensional genome structures of single sensory neurons in mouse visual and olfactory systems. Nat Struct Mol Biol. 2019;26(4):297-307.
6. Schmitt AD, Hu M, Ren B. Genome-wide mapping and analysis of chromosome architecture. Nat Rev Mol Cell Biol. 2016;17(12):743-755.
7. Yu M, Ren B. The three-dimensional organization of mammalian genomes. Annu Rev Cell Dev Bi. 2017;33:265-289.
8. Kong S, Li Q, Zhang G, Li Q, Huang Q, Huang L, et al. Exonuclease combinations reduce noises in 3D genomics technologies. Nucleic acids res. 2020;48(8):e44.
9. Liebermanaiden E, Van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, et al. Comprehensive mapping of long range interactions reveals folding principles of the human genome. Science. 2009;326(5950):289-293.
10. Rao SSP, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, et al. A 3D map of the human genome at kilo base resolution reveals principles of chromatin looping. Cell. 2014;159(7):1665-1680.
11. Nagano T, Lubling Y, Stevens TJ, Schoenfelder S, Yaffe E, Dean W, et al. Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. Nature. 2013;502(7469):59-64.
12. Tan L, Xing D, Chang C, Li H, Xie XS. Three-dimensional genome structures of single diploid human cells. Science. 2018;361(6405):924-928.
13. Nagano T, Lubling Y, Varnai C, Dudley C, Leung W, Baran Y, et al. Cell-cycle dynamics of chromosomal organization at single-cell resolution. Nature. 2017;547(7661):61-67.
14. Flyamer IM, Gassler J, Imakaev M, Brandao HB, Ulianov SV, Abdennur N, et al. Single-nucleus Hi-C reveals unique chromatin reorganization at oocyte-to-zygote transition. Nature. 2017;544(7648):110-114.
15. Stevens TJ, Lando D, Basu S, Atkinson LP, Cao Y, Lee SF, et al. 3D structures of individual mammalian genomes studied by single-cell Hi-C. Nature. 2017;544:59-64.
16. Ramani V, Deng X, Qiu R, Gunderson KL, Steemers FJ, Disteche CM, et al. Massively multiplex single-cell Hi-C. Nature Methods. 2017;14(7648):263-266.
17. Ulianov SV, Tachibanakonwalski K, Razin SV. Single-cell Hi-C bridges microscopy and genome-wide sequencing approaches to study 3D chromatin organization. BioEssays. 2017;39(10):1700104.
18. Zheng M, Tian SZ, Capurso D, Kim M, Maurya R, Lee B, et al. Multiplex chromatin interactions with single-molecule precision. Nature. 2019;566(7745):558-562.
19. Rowley MJ, Nichols MH, Lyu X, Andokuri M, Rivera ISM, Hermetz KE, et al. Evolutionarily conserved principles predict 3d chromatin organization. Molecular Cell. 2017;67(5):837.
20. Zhang Y, Wong C, Birnbaum RY, Li G, Favaro R, Ngan CY, et al. Chromatin connectivity maps reveal dynamic promoter–enhancer long-range associations. Nature. 2013;504(7479):306-310.
21. Ou HD, Phan S, Deerinck TJ, Thor A, Ellisman MH, O'Shea CC. ChromEMT: Visualizing 3D chromatin structure and compaction in interphase and mitotic cells. Science. 2017;357(6349):e0025.
22. Dixon JR, Gorkin DU, Ren B. Chromatin domains: the unit of chromosome organization. Mol Cell. 2016;62(5):668-680.