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Research Article

NOVEL PERSPECTIVE FOR VALIDATING EXISTENCE OF DNA-TO-DNA TRANSCRIPTION IN PLASMODIUM FALCIPARUM

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Abstract

In 2016, I published an article named as 'certain amplified genomic-DNA fragments (AGFs) may be involved in cell cycle progression and chloroquine Is found to induce the production of cell-cycle-associated AGFs (CAGFs)'. I assumed that the mechanism of production of CAGFs is via DNA-to-DNA transcription that uses DNA polymerase instead of RNA polymerase. Ten years have passed since I published this paper, but no researchers were interested in this topic. Recently, researchers from Kyoto University using super-resolution lattice-structured illumination microscopy (lattice-SIM) have discovered a high abundance of single-stranded DNA (ssDNA) beyond the 'outer shell' of the nucleolus，which indirectly supports my hypothesis and provides a good method of using Lattice-SIM for viewing ssDNA molecules. Learning from this method, a novel perspective to validate existence of DNA-to-DNA transcription in *Plasmodium falciparum* is presented in this paper.

Keywords: DNA-to-DNA transcription, *Plasmodium falciparum*, Lattice structured illumination microscopy (Lattice-SIM), Polymerase chain reaction (PCR), Single-stranded DNA (ssDNA).

INTRODUCTION

DNA stores all genetic information in all living cells, which can be transcribed and translated into vinous proteins that are required for all cellular functions. Therefore, DNA to RNA transcription is vital important because all the RNAs are produced by transcription. For example, rRNA, mRNAs and non-coding RNAs including long non-coding RNAs, microRNAs and small RNAs are produced through RNA polymerases (Pol I, Pol II, and Pol III) transcription [1]. Some non-coding RNAs are involved in regulation of gene expression or could be structural RNAs [2]. Interestingly, single-stranded DNA (ssDNA) is quite like RNA because both ssDNA and RNA need DNA template to generate themself. If non-coding RNA could be regulatory or structural RNA, why ssDNA cannot be regulatory or structural ssDNA? Recently. Researchers from Kyoto University using super-resolution lattice-structured illumination microscopy (lattice-SIM) have discovered high abundance of ssDNA beyond the 'outer shell' of the nucleolus, assuming that ssDNA forms a molecular complex with histone H1 for nucleolar encapsulation, which indicates that ssDNA could have structural function [3]. In 2016, I published an article named as 'certain amplified genomic-DNA fragments (AGFs) may be involved in cell cycle progression and chloroquine (CQ) Is found to induce the production of cell-cycle-associated AGFs (CAGFs)'. I assumed that CAGFs might be ssDNA molecules produced via DNA-to-DNA transcription that uses DNA polymerase instead of RNA polymerase and like cyclins, CAGFs might be regulatory molecules involved in regulation of cell-cycle progression [4]. Hereafter, four articles about generation of ssDNA that might be linked to DNA-to-DNA transcription were published [5]-[8]. Research findings from Kyoto University indirectly supports my DNA-to-DNA transcription hypothesis. Learning from the method used by researchers from Kyoto University, a novel perspective for validating existence of DNA-to-DNA transcription in *Plasmodium falciparum* is presented in this paper.

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POLYMERASE CHAIN REACTION (PCR) DETECTION AND LATTICE-SIM OBSERVATION OF SSDNA MOLECULES

In the article [4], two assumed-ssDNA bands of UB1- CAGF and UB2- CAGF have been reported. UB1-CAGF has been sequenced and two specific primers SP1(5'-AATAAGATGTGCTGTTTAACT-3') and SP2(5'-GTTGTGCTCACACTTATCT-3') have been reported as well in the paper for repeating the results by interested researchers. Both *P。falciparum* CQ-resistant K1 isolate and *P. falciparum* CQ-sensitive HB3 isolate were treated by CQ at concentration of IC10 (K1= 25nM and HB3 = 5nM) and their samples were collected at different time of cell-cycle (2h, 6h, 12h, and 24 h) could be detected by PCR with these two specific primers. At the same time, the nucleus of plasmodium cells could be viewed with lattice structured illumination microscopy (Lattice-SIM). Assumedly, the PCR bands could be big or small at different time collected samples. If Lettice-SIM observation results correspond well with the PCR bands, the DNA-to-DNA transcription in *P. falciparum* could be partially resolved. I am retired and have no resources to carry out the experiment except providing a schematic diagram for interested researchers to conduct the experiment (**Figure 1**), and the PCR band size is an assumed size based on the figure-1 results published in the article [4]. Putting it all together, I might conclude that this schematic diagram represents a novel perspective for validating existence of DNA-to-DNA transcription in *P. falciparum*. CAGFs that are assumed to be ssDNAs were induced by CQ, which indicates that ssDNAs are quite like RNAs because RNAs are uplifted as a stress response following genotoxic drug treatment [9][10]. If ssDNAs are dynamic during cell-cycle progression, they could function like cyclins-dependent protein kinases in regulation of cell-cycle progression [11].

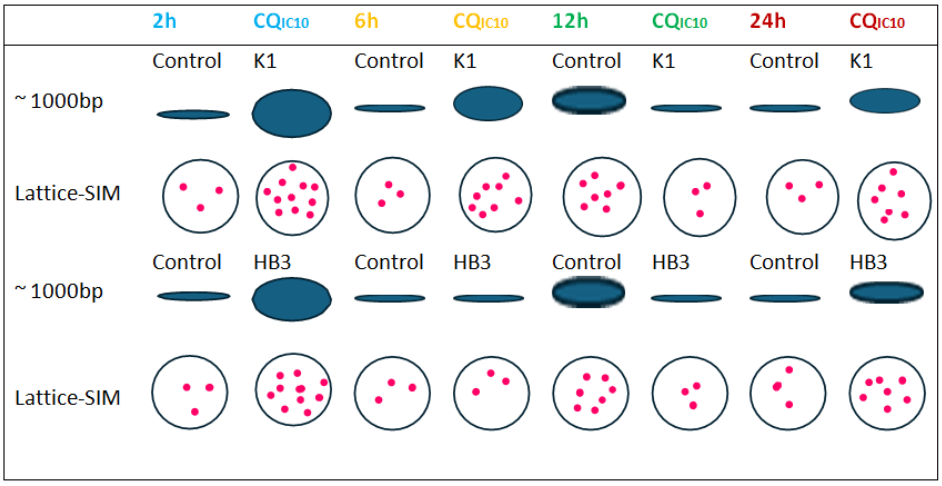

Figure 1. Schematic diagram of PCR detection and microscopic observation of CQ-resistant K1 and CQ-sensitive HB3 isolates. Both K1 and HB3 isolates were treated with CQ at concentration of IC10 (K1=25nM, HB3=5nM). CQ treated samples and control samples collected at different intervals (2h, 6h, 12h, and 24h) are detected with PCR using two specific primers (Sp1 and Sp2) and the PCR band length is about 1000 bp. Lattice structured illumination microscopy (Lattice-SIM) will be used to view and count the numbers of ssDNA molecules in the nucleus of plasmodium cells. Red dotes represent numbers of ssDNA molecules

Since ssDNAs are not proteins, they might be involved in regulation of gene expression, and formation of certain macromolecular complexes required for the dynamic genome architecture during cell-cycle progression [4]. If above two experiments has proved CAGFs to be ssDNA molecules, the DNA-to-DNA transcription could be partially solved because 'DNA-to-DNA transcription bubble' has not been found and 'DNA polymerase complex' that transcribes ssDNA transcripts has not been identified. I assume that in eukaryotic cells more than 90% DNA transcription belongs to DNA-to-RNA transcription and less than 10% DNA transcription might belong to DNA-to-DNA transcription. This might be the reason why DNA-to-DNA transcription is difficult to establish. Therefore, to prove existence of DNA-to-DNA transcription in eukaryotic cells, more hard work is ahead, needing long-time to identify 'DNA polymerase complex' that transcribes ssDNA transcripts and to find 'DNA-to-DNA transcription bubble structure' especially in non-coding DNA regions.

CONCLUSION

In 2016, I published an article that indicated existence of DNA-to-DNA transcription in *P. falciparum*. To make researchers repeating the results easily, two specific primers (SP1 and SP2) were published as well in the article [4]. Ten years have passed since I published this paper, but no researchers were interested in this topic. Recently, researchers from Kyoto University using super-resolution lattice-structured illumination microscopy (Lattice-SIM) have discovered a high abundance of ssDNA beyond the 'outer shell' of the nucleolus [3], which indirectly supports my hypothesis. I think that researchers can detect UB1-CAGF in *P, falciparum* by PCR with SP1 and SP2 and use Lattice-SIM to view and count ssDNA molecules in CQ treated samples and control samples that are collected at different time (2h, 6h, 12h, and 24h). If the results from the two methods match the expectations, validation of DNA-to-DNA transcription in *P. falciparum* could be partially solved, which is a novel perspective at this moment. The next goal is to identify 'DNA polymerase complex' that transcribes ssDNA transcripts and to find 'transcription bubble structure', especially in non-coding DNA regions.

CONFLICTS OF INTEREST

The author declares no conflicts of interest.

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