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Research Article

AMPLIFIED GENOMIC-DNA FRAGMENTS (AGFS) PRODUCED IN PLASMODIUM FALCIPARUM MIGHT BE SINGLE-STRANDED DNA MOLECULES RELEASED FROM DNA-TO-DNA TRANSCRIPTION

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Abstract

I published an article in 2016, the findings of which had shown that K1 CQ-resistant and HB3 CQ-sensitive isolates of *Plasmodium falciparum* might produce AGFs that were renamed as CAGFs because they were associated with cell cycle. The CAGFs could be induced by chloroquine (CQ) and appeared and disappeared during cell cycle progression, which suggested that CAGFs could be single-stranded DNA (ssDNA) molecules that might be produced via DNA-to-DNA transcription. There were two discovered CAGFs that were named as UB1 and UB2. I cloned and sequenced UB1 and designed two specific primers for PCR detection of UB1 in all samples that were previously detected by arbitrarily-primed PCR. Luckly. I obtained a specific PCR band from K1 2h-sample, but I couldn't carry on the research further to check all samples with specific PCR due to some uncontrolled factors. After carefully analysing the K1 2h-sample specific PCR band and its corresponding arbitrarily-primed PCR picture, I realized that the specific PCR band in all samples could be deduced based on the bands of arbitrarily-primed PCR. The figures in this paper have shown the results of deduced UB1 specific PCR band in all samples, which might help to confirm the research findings that CAGFs or ssDNA molecules were discovered by arbitrarily-primed PCR.

Keywords: Amplified Genomic-DNA Fragments (AGFs), Cell-Cycle-Associated AGFs (CAGFs), *Plasmodium falciparum*, Chloroquine, Single-Stranded DNA (ssDNA), DNA-to-DNA transcription, Arbitrarily-primed PCR.

INTRODUCTION

Using arbitrarily-primed PCR, amplified genomic-DNA fragments (AGFs) was found and induced by chloroquine (CQ) in K1 CQ-resistant and HB3 CQ-sensitive isolates of *Plasmodium falciparum*. Since AGF was associated with cell cycle, the AGF was renamed as CAGF. Two CAGFs were found, which are named as UB1 and UB2, respectively [1]. I cloned and sequenced UB1 and designed two specific primers for conducting specific PCR. The best results of UB1 specific-PCR band in K! sample collected at 2h were obtained and a photo of these results was taken. I felt happy because I thought them being a great discovery. But some experts thought that these arbitrary-primed PCR bands could be artifacts and no value for publishing, which discouraged me a lot. At that time, my contract was nearly finished, I needed to find a new job and I would leave the laboratory in couple of weeks. Therefore, the work had not been finished, and no other samples were detected with specific PCR. The experiments were conducted by me in the University of Liverpool around late 1998 to early 1999. After 17 years, I started to write manuscript of this article because I had firmly believed that these findings could be great discovery that must be reported. Unluckily, the photo of K1 2-h sample specific PCR band was lost, which is the reason why I included two specific primers (SP1 and SP2) in the article, but no picture was shown. Now 10 years has passed since the article was published, but no interested researchers cited or repeat the results, the reason of which could be no specific PCR results in all samples. Thus, in this paper, I present schematic diagram of deduced UB1 specific-PCR band in all samples that were missed portion in the published article [1].

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SCHEMATIC DIAGRAM OF DEDUCED UB1 SPECIFIC-PCR BAND IN ALL SAMPLES THAT WERE PREVIOUSLY DETECTED BY ARBITRARILY-PRIMED PCR

The research findings of arbitrarily-primed PCR were reported in published article [1], which indicated that K1 CQ-resistant and HB3 CQ-sensitive isolates of *P. falciparum* might produce amplified genomic-DNA fragments (AGFs) that were renamed as CAGFs because they were associated with cell cycle. The two CAGFs named as UB1 and UB2 were induced by CQ. UB1 was cloned and sequenced and two specific primers were ordered for detecting UB1 in all the samples by specific PCR. The two primers are SP1 (5'-AATAAGATGTGCTGTTTAACT-3') and SP2 (5'-GTTGTGCTCACACTTATCT-3'). If specific PCR using SP1 and SP2 primers could clearly confirm UB1 results in all samples, the article will be interested by many researchers because these results indicate that UB1 could be single-stranded DNA (ssDNA) molecules that might be involved in regulation of cell cycle. Furthermore, both UB1 and UB2 was induced by CQ, which indicated that CAGFs might be produced as ssDNA molecules via DNA-to-DNA transcription that uses DNA polymerase instead of RNA polymerase [1][2].

At first, K1 isolate sample collected at 2h was detected by specific PCR using SP1 an SP2. The results were so good that I cannot believe it. Unfortunately, the photo of K1 2h-sample specific-PCR results had been lost when I was preparing manuscript for published article [1]. Now, redoing these specific PCR in all samples by me is impossible because of I am retired and have no resources available. But after carefully analysing the K1 2h-sample specific PCR band and it's corresponding arbitrarily-primed PCR bands, I realized that the specific PCR band in all samples could be deduced based on the results of arbitrarily-primed PCR [1]. The deduction rules are here: if no UB1 band was seen in arbitrarily-primed PCR,

small-sized UB1 band will appear in specific PCR; if UB1 band from unclear to clear one was seen in arbitrarily-primed PCR, big or much bigger-sized UB1 band will be seen in specific PCR. The mechanism of difference between arbitrarily-primed PCR and specific PCR is because efficiency of arbitrarily-primed PCR using one primer is much lower than specific PCR, which suggests that arbitrarily-primed PCR needs large number of templates to show UB1 band. Based on these analysis findings, all UB1 specific-PCR band in all samples could be deduced. In the following **Figure 1** and **Figure 2,** a picture of arbitrarily-primed PCR bands is put on the top [1] and deduced UB1 PCR band is located at bottom boxes so that readers can view linkage of the two PCR bands.

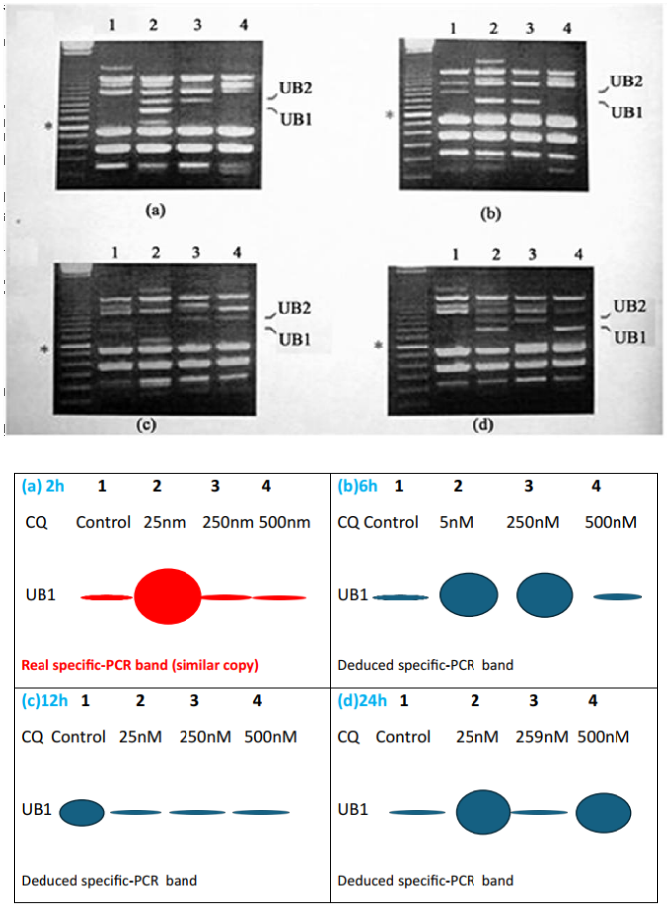
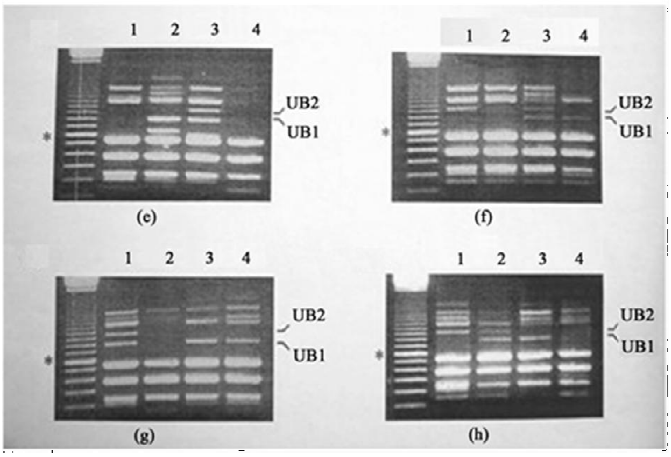


Figure 1. Schematic diagram of UB1 specific-PCR band seen in K1 isolate samples collected at 2h, 6h, 12h, and 24h. Top picture transferred from article [1], shows arbitrary primed PCR bands. The bottom box shows deduced UB1 specific-PCR band in all the samples. The length of UB1 specific-PCR band is about 1000 bp.



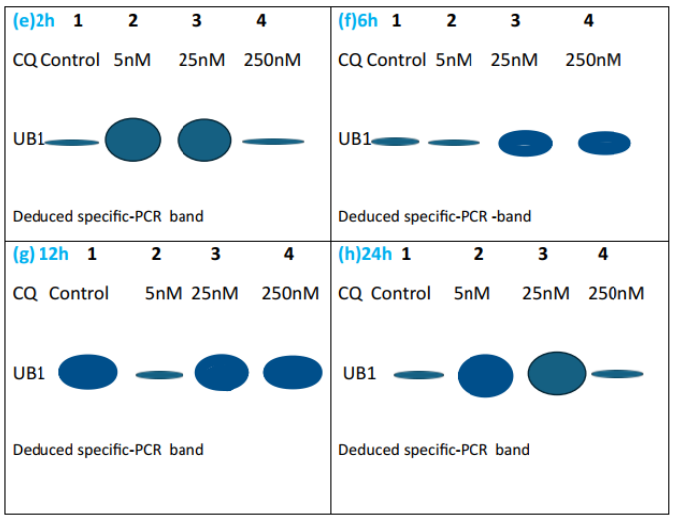


Figure 2. Schematic diagram of UB1 specific-PCR band seen in HB3 isolate samples collected at 2h, 6h, 12h, and 24h. Top picture transferred from article [1], shows arbitrary primed PCR bands. The bottom box shows deduced UB1 specific-PCR band in all the samples. The length of UB1 specific-PCR band is about 1000 bp.

Deduced UB1 specific-PCR band in all samples have proved that the findings obtained by arbitrarily-primed PCR are real facts but not artifacts. I think that publication of deduced UB1 specific-PCR band will help to confirm existence of CAGFS or ssDNA molecules in *P. falciparum*. I hope that interested researchers could repeat the results using SP1 and SP2 primers and *P. falciparum* model, which will promote CAGFs research in malaria parasite or help studying CAGFs in cancer cells. In control groups, the UB1 band in K1 and HB3 become bigger in the samples collected at 12h, which indicates that the number of PCR templates has naturally increased during cell cycle. This increase might be ssDNA molecules released from DNA-to-DNA transcription. Ther appearance and disappearance of CAGFs during cell cycle suggests that CAGFs could be ssDNA molecules that could be transcribed and decomposed, which quite likes cyclin-dependent protein kinases that regulate cell cycle [3]. It is obvious that CAGFs could be involved in regulation of cell cycle in *P. falciparum*. In addition, CAGFs induced by CQ might indicate that CAGFs could be stress response following genotoxic drug treatment that could be associated with CQ action. Extrachromosomal DNA (ecDNA) has been linked to many types of cancer and function as a drive for cancer developments [4][5]. I assume that ssDNA such as CAGFs released from DNA-to-DNA transcription could have certain relationship with ecDNA. Hopefully, CAGFs could play an important role in targeting cancer strategy.

CONCLUSION

Publication of schematic diagram of UB1 specific-PCR results has three reasons. First, UB1 and UB2 CAGFs that were discovered by arbitrarily-primed PCR in K1 CQ-resistant and HB3 CQ-sensitive isolates of *P. falciparum* could be real facts not artifacts. Second, as a supplementary data to the article [1], they would encourage interested researchers to repeat these results, which will promote ssDNA research. Third, *P. falciparum* could be a good model of eukaryotic cell for ssDNA study because CQ can induce CAGFs production in the cells. Researchers can design differently arbitrarily-primed PCR primers to check different samples during cell-cycle of

this parasite, and hopefully they could find more regulatory CAGFs or ssDNA molecules. Recently, researcher from Kyoto University using super-resolution lattice-structured illumination microscopy (Lattice-SIM) have discovered abundance of ssDNA in the nucleus [6]. I think that Lattice-SIM is an advanced technology that can help to rapidly find ssDNA molecules in the nucleus of eukaryotic cells. The novel perspective for validating existence of DNA-to-DNA transcription in *P. falciparum* was published recently, which suggested using the Lattice-SIM for observing ssDNA molecules [7]. I believe that application of Lattice-SIM will play an important role in the field of ssDNA exploration.

Arbitrary-primed PCR is usually used to fingerprint RNA and DNA between different cells for differential expression purpose [8] [9], but I used arbitrary-primed PCR to fingerprint genomic DNA from samples collected at different intervals of cell cycle, which is the unique idea to be able to discover CAGFs. I assumed that this CAGFs were ssDNA released from DNA-to-DNA transcription. The mainstream biological view and AI search have clearly denied my DNA-to-DNA transcription hypothesis, but my previous article‘s results [1] and this paper’s explored insights plus supporting findings of research from Kyoto University [6] might have proven existence of DNA-to-DNA transcription in *P. falciparum* or in all eukaryotic cells.

CONFLICTS OF INTEREST

The author declares no conflicts of interest.

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