PROTEIN-DNA CONJUGATE ARRAY CHIP FOR ON-CHIP DIRECTED EVOLUTION

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ABSTRACT

We have developed a new type of biomolecular array chip for high-throughput protein screening using cDNA display technology [1]. On the chip, mRNAs were arrayed via photo-crosslinking with a specially designed puromycin linker containing a photo-crosslinking reagent. Protein was synthesized in situ from the mRNA and immobilized on the chip via the puromycin linker. The combination of this array technology and our on-chip directed evolution system under development, by which 2.5x10^7 mutants can be assayed at the same time on a single chip [2], makes it possible to create functional proteins.

KEYWORDS: Protein chip, DNA chip, High throughput screening, directed evolution

INTRODUCTION

Directed evolution is a powerful approach to creating functional biomolecules and is expected to be performed more rapidly and effectively using a microchip device. In the directed evolution of proteins, the assignment of genotype (DNA/mRNA) and phenotype (protein) is essential. Protein-cDNA/mRNA conjugation via a puromycin linker (cDNA display) is a useful assigning technology to create functional proteins [1]. However, it has not yet been carried out on a microchip device. Therefore, we have developed a protein-cDNA/mRNA conjugate array chip to perform the on-chip directed evolution of proteins.

Figure 1 shows a simplified schematic of the on-chip directed evolution of proteins. Firstly, mutant mRNAs are distributed on the chip. Secondly, mutant proteins and cDNAs are synthesized in situ from the mutant mRNAs and immobilized on the chip. Thirdly, functions of the mutant proteins are evaluated. Finally, the cDNAs of highly functional proteins are collected from the chip followed by amplification and mutation to prepare the mutant mRNAs for the next round of on-chip selection. These subsequent on-chip selection rounds allow us to create desired functional proteins. Through these procedures, the preparation of a mutant library, the distribution of mutant mRNAs and the in situ synthesis of mutant proteins on a chip have been achieved [2]. Additionally, the collection of cDNAs from a chip has also been achieved [3]. In this study, technology for the immobilization of a protein and its cDNA/mRNA on the same spot has been developed.

Figure 1. Simplified schematic of directed evolution of proteins using microchip device. Functional proteins are evolved by performing subsequent selection rounds.
EXPERIMENTAL

Figure 2 shows the experimental procedure for the immobilization of protein-mRNA conjugates. A puromycin linker composed of a photo-crosslinking reagent, terminal thiol group and DNA was synthesized by general phosphoramidite chemistry and immobilized on a sputtered gold surface. Mixtures with different ratios of Cy5-modified and unmodified mRNA were spotted on the gold surface and hybridized with the puromycin linkers immobilized on the surface. After washing away the excess unhybridized mRNA, ultraviolet (UV) irradiation was performed on the whole surface to photo-crosslink the mRNA and the puromycin linkers followed by observation of the photo-crosslinked Cy5-modified mRNA. A Typhoon 9410 fluorescence scanner (GE Healthcare; excitation: 533 nm, emission: 580BP30 nm) was used. Subsequently, Rabbit Reticulocyte Lysate (Retic Lysate IVT Kit, Ambion) supplemented with FluoroTect GreenLys in vitro Translation Labeling system (Promega) was loaded on the surface and incubated to translate the proteins. The translated proteins were spontaneously immobilized on the surface via the puromycin linkers. After the translation reaction, the surface was washed with magnesium-containing solution and fluorescence images of Cy5-mRNA and FluoroTect-modified proteins were observed on the surface on which Cy5-mRNA was immobilized (Figure 3B). However, after washing with solution without magnesium, the signal of FluoroTect-modified proteins was decreased (Figure 3C). These results indicate that many proteins were synthesized and immobilized on the mRNA in polyribosome form in the presence of magnesium (Figure 3B), and the polyribosome and proteins were dissociated in the absence of magnesium and only the proteins, which were immobilized via puromycin linkers, remained on the surface (Figure 3C).

RESULTS AND DISCUSSION

Figure 3 shows the results of the immobilization of Cy5-mRNA via photo-crosslinking and the immobilization of the proteins via puromycin linkers. As shown in Figure 3A, Cy5-mRNA was successfully immobilized on the surface after UV irradiation. After translation and washing with magnesium-containing solution, FluoroTect-modified proteins were observed on the spots on which Cy5-mRNA was also immobilized (Figure 3B). However, after subsequent washing with a solution without magnesium, the signal of FluoroTect-modified proteins was decreased (Figure 3C). These results indicate that many proteins were synthesized and immobilized on the mRNA in polyribosome form in the presence of magnesium (Figure 3B), and the polyribosome and proteins were dissociated in the absence of magnesium and only the proteins, which were immobilized via puromycin linkers, remained on the surface (Figure 3C).
CONCLUSION
The technology to fabricate a protein-cDNA-mRNA conjugate array chip has been developed. mRNA was immobilized on a chip via photo-crosslinking with a specially designed puromycin linker, which was previously immobilized on the chip. Subsequently, protein was synthesized on the chip using the mRNA and a cell-free translation system and the protein was immobilized on the chip via the puromycin linker to form the protein-mRNA conjugate array chip. This technology is expected to be used for the on-chip directed evolution of proteins.

ACKNOWLEDGEMENTS
This research is supported by the Core Research for Evolutional Science and Technology (CREST) program of the Japan Science and Technology Agency (JST).

REFERENCES

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