Title	Study on Role of Liposome Membrane on Gene Expression of GFP in E.coli Cell-Free Translation System
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Citation	大阪大学, 2009, 博士論文
Version Type	VoR
URL	https://hdl.handle.net/11094/49613
rights	
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博士の専攻分野の名称	博　士（工　学）
学　位　記　番　号	第　２３００１　号
学位授与年月日	平成 21 年 3 月 24 日
学位授与の要件	学位規則第４条第１項該当 基礎工学研究科物質創成専攻
学　位　論　文　名	Study on Role of Liposome Membrane on Gene Expression of GFP in *E.coli* Cell-Free Translation System （大腸菌由来無細胞タンパク質合成系における GFP の遺伝子発現におよぼすリポソーム膜の役割に関する研究）
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論　文　内　容　の　要　旨

Liposome plays an important role in application of drug delivery and can enhance gene delivery and expression inside the cell. However, the role and function of liposome to enhance gene delivery and promote gene expression were still not yet clarified. In this study, the possible role and function of various kinds of liposome on gene expression and their mechanism were investigated and clarified at elementary step of the gene expression of Green fluorescence protein (GFP) such as transcription, translation and folding.

Chapter I: Role of Biomembrane in Gene Expression through Analysis of Elementary Steps: The effect of some kinds of liposome to enhance gene expression of a reporter protein, green fluorescence protein (GFP), was investigated through the investigation of the elementary steps of the gene expression, such as transcription, translation and folding. The result indicated that GFP product was enhanced when it was expressed in the presence of liposome prepared with the 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidyl chorine (POPC) and cholesterol (Ch). The amounts of mRNA were found to be promoted and higher than those in the control sample. However, negative and positive charged liposome inhibited gene expression. The above results show that the POPC/Ch liposome could primarily affect the transcription of GFP gene among the possible steps of gene expression, resulting in the enhanced GFP expression.

Chapter II: Role of Model Biomembrane on Gene Expression ~ Negative Charged Liposome Effect on *in vitro* Gene Expression~: The role of negatively-charged liposome prepared by (POPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG) on GFP expression was investigated. The results indicated that, in the presence of liposome, the GFP fluorescence was reduced although total product of synthesized GFP was not changed. The above results indicated that the POPC/POPG liposome could inhibit the GFP expression at a post-translational process (folding) of GFP during its gene expression. The liposome also could strongly interact and inhibit the refolding of unfolded state of GFP to its native one. In this case, surface and and interior membrane fluidity of liposome was reduced significantly, implying that the liposome could interact with polypeptide GFP by electrostatic attraction and then interact strongly by hydrophobic interaction to give an inhibition of folding of expressed GFP and refolding of unfold native GFP.

Chapter III: Role of Foreign Membrane on Gene Expression ~ Effect of Cationic Liposome and Stressed Liposome on Gene Expression at Transcription and Translation level~: The effect of cationic liposome on inhibition of gene expression and its mechanism was investigated. The result indicated that 1,2-dioleoyl-3-trimethyl- ammonium-propane (DOTAP) liposome can knockout gene for inhibition of gene transcription. This result implied that liposome could strongly interact with DNA by electrostatic attraction between cationic amino groups in liposome and negative charged phosphate groups of DNA, and/or by hydrogen-bond interaction. Furthermore, cationic liposome can also knock-down and interfere mRNA translation. In the presence of DOTAP liposome, mRNA could still exist although it could not be translated into protein. The results indicated that cationic amino groups of liposome can strongly interact with negative charged phosphate groups of mRNA, and neutralized mRNA, resulted in interference of mRNA and silenced its translation. DOTAP liposome can be applied as a new drug modified with specific ligand (ex: ESA (for interaction with target cell (ex cancer cell), inhibition of its growth by knockout gene and/or knockdown mRNA inside the cell.

Chapter IV: Gene Expression Regulated by Membrane and Stress ~ Gene Expression in vitro Regulated by Stressed Liposome and Deeper Understanding of in vivo Gene Silence ~: The role of the liposome membrane to promote and inhibit the gene expression systems has been summarized to establish the strategy to regulate the gene expression. First, the spectra of the gene regulation by the membrane were summarized with respect to the elementary steps included in the gene expression system, such as (i) transcription, (ii) translation, and (iii) folding. A possible regulation of the gene expression has been achieved by employing some case studies, such as (1) proposal on the GFP production regulated by liposome membrane, (2) re-evaluation of the gene silence of cry protein in *Bacillus thuringiensis* cells, and (3) stress-mediated regulation of GFP gene on the liposome membrane in the cell-free translation system.

The obtained results on the strategy for the regulation of the gene expression by the stressed membrane (membrane interference) were summarized in general conclusion together with the suggestions for future works focusing on the gene silencing and/or bioprocess design.

List of Publications

[Original Papers]

1. Huong Thi Bui, Hiroshi Umakoshi, Kien Xuan Ngo, Masato Nishida, Toshinori Shimanouchi, and Ryoichi Kuboi; Liposome membrane its self can affect Gene Expression in *E.coli* Cell-Free Translation System, *Langmuir,* **24**, 10537-10542 (2008).
2. Huong Thi Bui, Khang Duy Dinh, Chau Thuy Nguyen: Cloning and Sequencing Specific DNA Fragment of cryIAb Gene from Some *Bacillus thuringiensis* var. *kurstaki* strains isolated in Vietnam. *Genetics and Application*, **4**, 1-7 (2003). ISSN: 0866-8566.
3. Huong Thi Bui, Khang Duy Dinh, Chau Thuy Nguyen: Some Biochemical and Biomolecular Characteristics of *Bacillus thuringiensis* var. *aizawai* H1 strain isolated in Vietnam. *Genetics and Application*, **3**, 1-7 (2003). ISSN: 0866-8566.
4. Huong Thi Bui, Hiroshi Umakoshi, Keishi Suga, Tomoyuki Tanabe, Toshinori Shimanouchi, and Ryoichi Kuboi: Cationic Liposome Inhibit Gene Expression in *E.coli* Cell-Free Translation System; *Membrane (Maku),* accepted.
5. Huong Thi Bui, Hiroshi Umakoshi, Keishi Suga, Masato Nishida, Toshinori Shimanouchi, and Ryoichi Kuboi: Negative-Charged Liposome Is Potent Inhibitor of Post-Translation During *in vitro* Synthesis of Green Fluorescence Protein, *Biochemical Engineering Journal,* submitted.

[Related Papers]

1. Kien Xuan Ngo, Hiroshi Umakoshi, Toshinori Shimanouchi, Huong Thi Bui and Ryoichi Kuboi. Active Roles of liposomes and Heat Stress for Enhanced Realease of Chitosanase from Streptomyces griseus, *Journal of Bioscience and Bioengineering* **106(6)**, 602-605 (2008).
2. Huong Thi Vu, Huong Thi Bui, Nguyen Thuuy Chau, Cloning and sequencing MetA gene, a methionine biosynthetic gene encoding Homoserin acetyltransferase enzyme from isolates of *Corynebacterium glutamicum* in Vietnam. *Journal of Biotechnology*, in press
3. Huong Thi Bui, Khang Duy Dinh, Huyen Ngoc Do Thi, Tuan Nguyen, Chau Thuy Nguyen: Isolation and Characterization some *Bacillus thuringiensis* var. *kurstaki* Strains in Vietnam. *Genetics and Application*, **3**, 8-14 (2003). ISSN: 0866-8566
4. Huyen Thi Ngoc Do, Huong Thi Bui, Khang Duy Dinh, Tuan Nguyen, Chau Thuy Nguyen, Michio Ohba; Characterization of Vietnam isolates of *Bacillus thuringiensis* serovar *kurstaki* effective for control of insect pests. *Genetics and Application*, **4**, 12-16 (2002). ISSN: 0866-8566.
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6. Huong Thi Bui, Khang Duy Dinh, Huyen Thi Ngoc Do, Tuan Nguyen, Chau Thuy Nguyen, Tuan Van Tran, Huyen Ngoc Nguyen, Ha Hong Nguyen, Hieu Huu Le: Researches on the technology of producing microbial insecticide by use of *B. thuringiensis* to control insects. *Journal of Science Technology and Economic Management,* **8**, 359-361 (2000). ISSN: 0866-7020.

[Proceedings]

1. H.T.Bui, D.N.Huyen, D.D.Khang, N.T.Chau, Some biochemical and biomolecular characteristic of *Bacillus thuringiensis* strains isolated in Vietnam, *Proc. of 5th Pacific Rim Conference on the Biotechnology of Bacillus thuringiensis and its Environmental Impact*, 17-21 November, p. 129-135, Hanoi, Vietnam (2003)
2. N.T.Chau, H.T.Bui, D.T.N.Huyen, T.V.Tuan, N.N.Huyen, N, H. Ha, N. Tuan, N. D. Tien, L. H. Hieu, Production technology of bioinsecticide *Bacillus thuringiensis* in the fermentation system 1500L/batch and its application to control lepidopteran and coleopteran insects in vegetable, fruit and stored food grain, *Proc. of 4th Pacific Rim Conference on the Biotechnology of Bacillus thuringiensis and its Environment Impacts,* 11- 15 November, p.210-214, Canberra, Australia (2001).
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5. V.K.Thoa, H.T.Bui, N.T.Chau, Study on the production technology of *Ganoderma lucidum* biomass. *Proc. of the 8th Asean Food Conf.,* 8-11 October, Hanoi, Vietnam. Vol 2, 703-708 (2003)
6. H.T. Bui, K.X. Ngo, H. T.Vu, K. D. Dinh, C. T. Nguyen; Isolation of *Corynebacterium glutamicum* Strains and Cloning of met A, a Methionine Biosynthetic Gene Encoding Homoserin Acetyltransferase from Soil in Vietnam. *Proc. of 3rd Int'l Symp. on Engineering Science of Liposome and Membrane Stress Biotechnology,* November, 3-4, Toyonaka, Osaka (2005).
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8. H. T. Bui, H. Umakoshi, K. X. Ngo, T. Shimanouchi, R. Kuboi. Enhancement of Protein Synthesis by Liposome in *E.coli* Cell-Free Translation System. *Proc. of 8th International 21st Century COE Symposium on Integrated EcoChemistry (COEIEC VIII),* Kyoto, Japan. August 28-29 (2006).
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10. H. T. Bui, H. Umakoshi, K. Suga, T. Shimanouchi, R. Kuboi. Cationic Liposome can Knock-down mRNA and Silence Its Translation in *E.coli* cell free Translation System. *Proc.of JSPS-SNSF International Conference on Membranomics*, Osaka University, Toyonaka (2008)

論 文 審 査 の 結 果 の 要 旨

生命現象の本質的な理解のために，ゲノム(Genome)およびプロテオーム(Proteome)というアプローチが一般的である．しかし，上記の既存ome情報のみでは根本的な理解は難しく，例えば，遺伝型(genotype)と表現型(phenotype)の違いの説明が難しい点も指摘されている．近年，ストレス条件下で顕在化される生体膜自身の潜在機能に着目したメンブレノーム(Membranome)という概念が提案されている．しかし，遺伝子発現プロセスにおける生体膜の役割は明らかにされていないのが現状である．

本学位論文では，無細胞タンパク質合成系におけるモデルタンパク質(Green Fluorescent Protein: GFP)の遺伝子発現挙動が，ストレス負荷(モデル)生体膜(リポソーム)の潜在機能により，促進・阻害されるという現象について体系的に検討された．さらには，上記の基礎原理に基づいて(i)生体細胞内の遺伝子抑制効果の考察ならびに(ii)無細胞タンパク質合成系におけるGFP発現のストレス応答制御に応用した．１章では，無細胞タンパク質合成系における各種リポソーム添加効果について検討されている．遺伝子発現プロセスを(i)転写過程，(ii)翻訳過程，(iii)フォールディング過程の３つの素過程として捉え直し，リポソームが素過程に及ぼす影響について解析する手法を確立し，GFPの生産量を改善する条件を明らかにした．２章では，生体膜に多く含まれる負電荷脂質からなるリポソームがGFP発現挙動に及ぼす影響が検討されている．負電荷リポソームの場合，転写・翻訳過程に及ぼす影響は少なく，タンパク質の特性依存的にフォールディング過程に強く影響する事が示されている．３章では，遺伝子送達技術(DDS)などで使用される人工的な脂質の効果について検討されている．上述の手法を用いて，DOTAPといわれる正電荷を持つリポソームがGFP遺伝子発現に及ぼす影響を解析し，DOTAPリポソームが，遺伝子との相互作用により，転写・翻訳過程を強く阻害する事を示されている．４章では，上記の知見に基づいて，モデル生体膜の特性とGFP遺伝子発現素過程との関連性が体系的に示されており，生体膜を用いた遺伝子発現の制御の可能性が示唆されている．ケーススタディとして，(i) グラム陽性菌変異体におけるモデルタンパク質(cryタンパク質)の発現抑制効果の考察，(ii) 無細胞タンパク質合成系における各種タンパク質の発現制御の提案，(iii) 無細胞タンパク質におけるDOTAPリポソームのGFP遺伝子抑制効果の制御が検討された．

以上の様に，本学位論文では，無細胞タンパク質合成系におけるモデル生体膜の役割に関する体系的に検討を進めると同時に，ストレス制御型遺伝子発現の制御手法についても示されている．よって，博士（工学）の学位論文として価値のあるものと認める．