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温度感受性変異株を補い、変異 Na⁺/H⁺ antiporter の IPTG 誘導に感受性を与える大腸菌遺伝子の選択

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Membrane integrated proteins play significant roles in cell's life from bacteria to human. They transport different types of chemical compounds into or out of cells with complicated regulatory networks. If their functions are disrupted, the life should be lost. Recently, three-dimensional structure analysis revealed astonishing complicated structures of many membrane proteins. What kind of machinery constructs those varieties of membrane proteins? We know that the Sec machinery works central roles in membrane proteins' insertion in membranes, from bacteria to human. We like to know whether other new players may participate in membrane biogenesis or not. For this purpose, we tried to get genes that compliment ts (temperature sensitive) mutants which do not die in stead of induction of toxic membrane protein, hopefully induced extra toxic membrane protein is not integrated in those ts mutants' membranes effectively.

We found most *E. coli* strains died after IPTG addition if the cells had a plasmid pNhaBD147M (contains *bla* gene) carrying mutated Na⁺/H⁺ antiporter gene under *lac* promoter. We got 8 *E. coli* ts mutants from the Hirota collection, which did not die under above condition. We made a bank of K12 chromosome using a vector pSTV28 (contains *cat* gene). We got 4 plasmids, pTS1 to 4, that complimented 42 °C growth of ts mutants, TS85, TS118, TS127, TS312, respectively. We failed to get plasmids that compliment ts phenotype of other 4 mutant strains. Each pTS plasmid makes respective ts mutant with pNhaBD147M die by addition of IPTG. Genes that cause ts phenotype and cell death by NhaBD147M induction of respective mutants were identified. We will discuss here what kinds of genes are responsible for this phenotype.

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