Department of Bacteriology

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General Summary

Research projects of our department have focused on: 1) role of gut microbe on host nitrogen cycle, 2) a novel single point mutation in domain 2 of the stress-inducible sigma factor RpoS attenuates its activity, 3) a straightforward assay for measuring glycogen levels and RpoS activity, 4) molecular mechanisms of curli biogenesis regulated by molecular chaperones, 5) roles of co-chaperones of Hsp70 in curli biogenesis, 6) staphylococcal biofilm dispersal via nuclease.

Research Activities

Role of gut microbe on host nitrogen cycle

Like oxygen, hydrogen, and carbon, nitrogen is an important element for the growth, maintenance, and survival of organisms. Nitrogen is abundantly present on earth; however, it predominantly exists in the air as molecular nitrogen, which is inactive and cannot be utilized by organisms. Compared to the amount of the bioavailable forms of other elements, the amount of bioavailable nitrogen can often be insufficient, and this insufficiency can restrict the increase in the biomass of organisms. We investigate roles of gut microbe on nitrogen cycle in host.

A novel single point mutation in domain 2 of the stress-inducible sigma factor RpoS attenuates its activity

RpoS is a sigma factor that regulates stress resistance genes in *Escherichia coli*, such as the *katE* encoding catalase HPII and the *glg* encoding glycogen synthesis proteins. Monitoring RpoS activity can provide information on the stress sensitivity of *E. coli* isolates in clinical settings because its RpoS is often mutated. In the present study, we found a novel, missense point mutation at the RpoS domain 2 in a clinical *E. coli* isolate. The mutant RpoS protein was non-functional according to the HPII activity and glycogen levels, which are positively regulated by RpoS. A reporter assay with β-galactosidase indicated that the dysfunction occurred at the transcriptional level. Substitution analysis indicated that the hydrophobicity of the amino acid at domain 2 was critical for RpoS activity. However, no RpoS activity was observed when RpoS domain 2 was substituted with the hydrophobic amino acid Pro, which can destroy the alpha-helix structure at the domain 2, suggesting that the structure near this residue may also play an important role in RpoS activity. These results contribute to a deeper understanding of RpoS regulatory mechanisms and bacterial stress responses.

A straightforward assay for measuring glycogen levels and RpoS activity
Bacterial cellular glycogen levels reflect the activity of RpoS. In this study, a straightforward assay for measuring glycogen levels and RpoS activity was developed combining the ease and simplicity of qualitative approaches. The basic principle of this assay is the iodine–glycogen reaction producing a reddish brown color that can be measured by spectrophotometer. The results indicate that the assay exhibited linearity within the range of standard solutions used (300 > μg/assay; $R^2 = 0.994$) and that the minimum detected concentration of glycogen was 10 μg/100 μl per assay. The applicability of the assay was assessed; glycogen was detected and quantified in clinical isolates with functional RpoS but not in isolates with dysfunctional RpoS; this assay constitutes a simple method for measuring RpoS activity and was successfully applied for measuring glycogen levels in human cells.

Molecular mechanisms of curli biogenesis regulated by molecular chaperones
We discovered that curli biogenesis depends on molecular chaperone DnaK, a bacterial Hsp70 homolog, by undefined mechanism(s). In this study, we showed that DnaK positively regulated expression of CsgA and CsgB, the major and minor structural components of curli. In addition, biochemical and cell biological studies demonstrated that DnaK maintained a translocation competent state of CsgA by binding to the N-terminal aggregation-prone signal sequence, leading to successful translocation of CsgA into the periplasm. These results provide mechanistic insights underlying how DnaK regulates curli biogenesis and robust biofilm formation.

Roles of co-chaperones of Hsp70 in curli biogenesis
The nucleotide-regulated cycles of the 70 kDa heat shock proteins (Hsp70s) are controlled by co-chaperones, DnaJ-domain proteins (JDPs) and nucleotides exchange factors (NEFs). The bacterial major Hsp70 system consists of DnaK, three JDPs (DnaJ, CbpA, and DjlA), and one NEF (GrpE), but co-chaperones-independent functions of DnaK remain unresolved. Here, we show the dispensability of the co-chaperones for the DnaK-regulated formation of curli, extracellular amyloid fibers involved in biofilm formation and host colonization. Full specification of the DnaK system was essential for survival at high temperature, whereas either JDPs or GrpE was dispensable for the curli production. In addition, DnaK mutants with reduced activities strongly required both co-chaperones for curli biogenesis. These results suggest that activities of the Hsp70 system can differ among individual cellular functions.

Staphylococcal biofilm dispersal via nuclease
In the staphylococcal biofilm development, bacteria formed biofilm within 8 h, however, biofilm was dispersed after 24 h. Analysis of extracellular matrix of biofilm and culture supernatant showed that this dispersal correlated with nuclease which degrades nucleic acids in the matrix. The biofilm dispersal was not detected in *nuc* mutant, which indicated that nuclease is a key factor for biofilm disassembly mechanism. Induction of biofilm dispersal by nuclease depended on pH in the culture supernatant, suggesting that environmental pH is one of the cue signals for dispersal.

Publications

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