PD Research Report for the 2015 year

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Research Results （about 2,500 characters in Japanese, about 65 lines times 90 characters in English）

There were two major parts of our works in the last year. One is the study of cold-inducible archaeal chaperonin CpkA. *Thermococcus kodakarensis* grows optimally at 85°C and possesses two chaperonins, cold-inducible CpkA and heat-inducible CpkB, which are involved in adaptation to low and high temperatures, respectively. The two chaperonins share high sequence homology (77%), except in their C-terminal regions. CpkA, which contains tandem repeats of a "GGM" motif, shows highest ATPase activity at 60°C ~ 70°C, whereas in CpkB shows highest activity at temperatures higher than 90°C. To clarify the effects of changes in ATPase activity on chaperonin function at lower temperatures, various CpkA variants were constructed by introducing single point mutations into the C-terminal region. The CpkA mutant, in which Glu530 was replaced with Gly (CpkA-E530G), showed increased ATPase activity, with greatest activity at 50°C. The efficacy of the CpkA mutants against denatured indole-3-glycerol-phosphate synthase ($TrpC_{Tk}$), which is a CpkA target, was then examined in vitro. CpkA-E530G was more effective at facilitating the refolding of chemically unfolded TrpCTk at 50°C than wild-type CpkA. The effect of cpkA-E530G on cell growth was then examined by introducing *cpkA*-E530G into the genome of *T. kodakarensis* KU216 (*pyrF*). The mutant strain, DA4 (*pyrF*, *cpkA*-E530G), grew as well at 60°C as the parental KU216 strain. By contrast, DA4 grew more vigorously than KU216 at 50°C. These results suggested that the CpkA-E530G mutation prevented cold denaturation of proteins under cold-stress conditions, thereby enabling cells to grow in cooler environments. Thus, a single base pair substitution in a chaperonin gene allows cells to grow vigorously in a new environment.

The duplication and divergence of α and β chaperonin subunit genes such as cpkA and cpkB in *T. kodakarensis* were thought to have occurred in a common ancestor; the α subunit was subsequently lost from the Pyrococcus lineage. However, the results of the present study suggest another possibility. The natural habitat of Thermococcus is a solfatara, which fluctuate between middle (around solfatara, ~60°C) and high temperatures (at the center of solfatara, ~100°C); therefore, these thermophiles have evolved an extra chaperonin that allows them to adapt to a colder environment. Living in an environment that moves between temperature extremes facilitates natural selection; therefore, such thermophile can evolve quickly. Thus, Thermococci adapted to lower temperatures by duplicating the chaperonin subunit α (CpkA) which acts as an "adaptive allele" and enables *T. kodakarensis* to adapt to its changing habitat.
Cold tolerance and cold adaptation are related, but different, behaviors. Cold tolerance usually occurs in response to a temporary drop in temperature, whereupon cold shock proteins (CSPs) enable their hosts to survive. Cold adaptation refers to a longer period of low temperature, triggering a response by not only CSPs but also by many non-stress proteins. However, these non-stress proteins are unstable and incorrectly folded under cold-stress conditions. Chaperonin plays an important role in protein folding

and refolding; however, CpkB is not suited to this task under cold-stress conditions because it has low ATPase activity below 60°C. Thus, CpkA evolved as a unique cold-inducible chaperonin. CpkA and CpkB differ at the C-terminus, which forms part of equatorial domain. According to its C-terminal region, CpkA, a cold-inducible thermosome, was classified as a CpkA-type chaperonin belonging to group I chaperonins (which include GroEL of *E. coli*. By contrast, MM_1798, which encodes a mesophilic chaperonin, was classified as a CpkB-type chaperonin along with those thermosomes from Pyrococcus. Moreover, the growth temperature-dependent distribution of chaperonins and the growth profile of the *T. kodakarensis* disruptants of cpkA and cpkB suggests that CpkA-type chaperonins play a key role in cold adaptation. Without cpkA, *T. kodakarensis* disruptant DA1 was hard to grow at 60°C. An extra C-type chaperonin in microorganisms might respond to other situations; an example is the TCP1 (CCT)-containing chaperonin in eukaryotic cells, which has up to eight different subunits that interact with different substrates. The wide variation in the C-terminal regions of chaperonins enables microorganisms to adapt to changing environments.

In summary, we identified two growth temperature-dependent trends (correlation between the CpkA-type chaperonin gene copy number and growth temperature) in group II chaperonins, both of which are driven by the initial duplication and subsequent mutation of a chaperonin gene. The tandem repeats within the C-terminal region of CpkA are a mutational hotspot. Here, we showed that a single base pair substitution (E530G) in the C-terminal region of *T. kodakarensis* CpkA allowed the organism to adapt to a lower growth temperature (50°C). Because chaperonins regulate protein-protein interactions within cells, especially under stress conditions, changes in the activity of chaperonins will lead to changes in cell properties, such as cold adaptation. GroEL has a marked effect on the refolding of proteins called mutators, which are involved in DNA repair; thus, GroEL might indirectly increase the mutation rate of the host cell. Because GroEL and CpkA have a similar substrate bias, we would expect the role of CpkA in T. kodakarensis to resemble that of GroEL. Further *in vivo* studies should aim to derive a mesophilic lineage from hyperthermophiles to better understand cold adaptive mechanisms in archaea.

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The other work is about development of novel apparatus for protein trapping using thermostable chaperonin CpkB. Our purpose is to develop a new molecular apparatus to specifically recognize and stabilize proteins, especially heterologous proteins in vivo and in vitro. To achieve this goal, we constructed a chaperonin - labeled protein pair that chaperonin could specifically recognize and stabilize the target proteins through the signal-tail attached to the end of the proteins. Our plan is carried out as following route:

(1) Design and screen out the most efficient signal-tails which are recognized by chaperonins. It would take about 6 months.

(2) Optimize the chaperonin - signal-tail pair and their working condition. This would take about 3 months.

(3) Immobilize chaperonin. This would take about 3 months.

Through the procedure above, we would obtain a set of signal-tails which could be fussed into target protein and the labeled target proteins would be specifically recognized and stabilized by modified

Group II chaperonins — CpkA and CpkB. Using ATP analogs — BeF_x, the target proteins would be blocked selectively in the cavities of chaperonin from background proteins to avoid any cruel condition. By adding in excessive ATP, the target proteins would be released from chaperonin at last. We then could obtain the purified and functional target proteins in solution. We already obtained several functional signal-tails to enhance the specificity of CpkB.