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高度好熱菌由来 cold shock protein の「細胞内温度センサー」としての機能

“Intracellular thermosensor” function of a cold shock protein from *Thermus thermophilus* HB8

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All organisms have a biological system to enable them to adapt to an abrupt downshift in growth temperature, termed ‘cold shock’, which is one of the environmental stresses. This cold adaptation system exists to overcome the various negative influences caused by exposure to cold stress. Many proteins, termed ‘cold-induced proteins’ (CIPs), are induced by a temperature downshift although these proteins are repressed under normal growth conditions (Fig. 1). Several CIPs are required for various cellular activities including metabolism, transcription, translation and protein folding processes, that are affected by cold stress. The researches about cold shock response have been extensively performed. Then, several CIPs have been discovered by transcriptome analysis in various organisms. Because some CIPs directly function to overcome any negative influences caused by temperature downshift, it is possible to regard them as potential ‘thermosensor’ candidates. However, the cold shock response in terms of mRNA expression has been examined only over a time range of 10 min to several hours. In the initial stage of the cold shock response, therefore, very little is known about how the cell responds to temperature downshift. To elucidate the cold adaptation mechanism, it is necessary to investigate how cold sensing occurs at a very early stage immediately following cold shock.

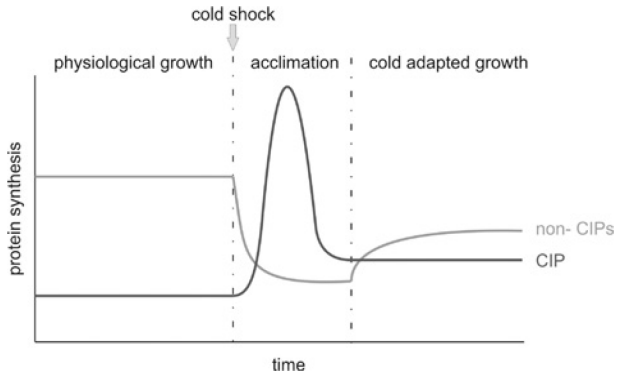

Fig. 1. Expression pattern of CIPs. (Cited from Horn, G. *et al.* (2007) *Cell. Mol. Life Sci.* **64**, 1457-1470)

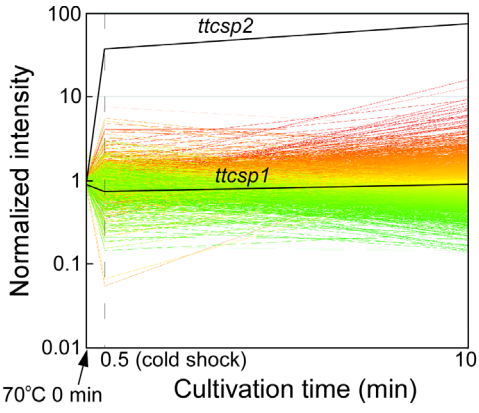

Fig. 2. Expression pattern of *ttcsp1* and *ttcsp2* obtained from DNA microarray.

To investigate the cold shock response on the initial stage of an extreme thermophile *Thermus thermophilus* HB8, we performed DNA microarray analysis. As a result, we found that *ttcsp2* mRNA was induced the most immediately after temperature downshift (from 70 to 45°C) in *T. thermophilus* HB8 (Fig. 2). This rapid induction of *ttcsp2* mRNA was verified by qRT-PCR. The mRNA expression level of *ttcsp2* increased 450-fold at 0.5 min and 770-fold at 10 min after cold shock. In contrast, the expression of *ttcsp1*, which is one of cold shock protein homologues, remained low and almost constant despite the temperature change. Therefore, we concluded that ttCSP1 and ttCSP2 are, respectively, cold-uninducible and cold-inducible CSPs of *T. thermophilus* HB8 at the transcriptional level. To investigate how this rapid increase of *ttcsp2* mRNA expression was induced, we determined its transcription start site and then predicted the secondary structure of the 5'-untranslated region (5'-UTR). Interestingly, its 5'-UTR formed one stem-loop structure at 70°C, but formed another stable stem-loop structure downstream the first one at 45°C (Fig. 3). This suggested that *ttcsp2* mRNA could function as a "thermosensor" to sense temperature downshift. We also performed western blotting to investigate the cold-inducibility of ttCSP2 at the translational level. Taken together with these results, we discuss the ttCSP2 expression induced by temperature downshift at both transcriptional and translational level. In addition, we also speculate about the molecular function of ttCSPs and the cellular interrelationship between ttCSP1 and ttCSP2.

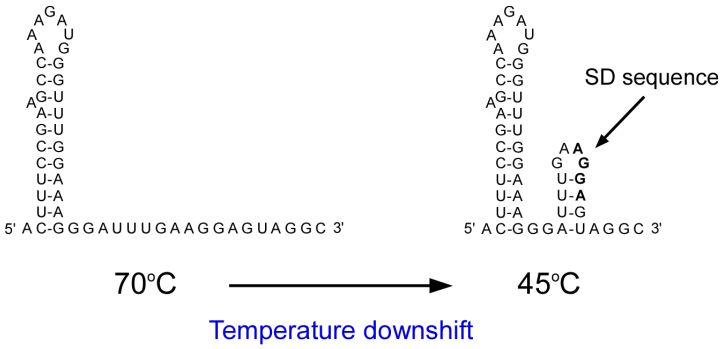

Fig. 3. The predicted secondary structure of *ttcsp2* mRNA in 5'-UTR at 70˚C (left) and 45˚C (right).

Reference

[1] Mega *et al.* (2010) *Biochem. Biophys. Res. Commun.* in press