Thermophiles are organisms that live at relatively high temperatures of at least 60°C. Studies on the survival mechanisms of these organisms have drawn great attention since the relevant knowledge helps us to understand how life can thrive under extreme temperatures, as well as the potential of thermophiles in biotechnology and whether they contain information regarding the early evolutionary life forms on Earth. Generally, microorganisms with an optimal growth temperature (OGT) between 60 and 80°C are designated as thermophiles, whereas those growing optimally at temperatures of >80°C are referred to as hyperthermophiles, which are found in the three domains of life, archaea, bacteria, and eukarya, but the majority are archaea and bacteria. As we focus on the molecular basis of the adaption of micro-organisms at high temperatures but not the biological taxonomy, thermophiles vs. hyperthermophiles and bacteria vs. archaea are not specifically differentiated and are simply denoted as thermophiles in this review.

Traditionally, most investigations have focused on the features of some certain molecules, such as the stability of protein structures or the enzyme activities of thermophiles. Based on many crystal structures of the reported thermophilic enzymes, several factors that are responsible for thermostability have been illustrated, such as a selection of amino acid substitutions (2), hydrophobic cores (10, 18), buried polar contacts and ion pairs (33), and interactions among subunits (59, 62). However, it is becoming a realization that, even though the thermo-tolerant mechanism for a single protein is completely elucidated, its biological significance is still only partially understood, because the life activity of a bacterium relies heavily on the coordination of the molecular networks within cells. Biological analyses using large-scale data are emerging in the field of thermophiles to explore the important thermophilic factors. A considerable number of studies have been conducted on the genome, transcriptome, and proteome, as well as the widespread and accumulated multiple-omics information concerning thermophiles, making it possible to systematically evaluate how these organisms survive under harsh thermal conditions.

In this review, we will summarize the findings extracted from “omics” data and discuss the role of omics studies on revealing thermophilic physiology. We pay less attention to the currently known single molecular adaptations for thermophiles.

The Genomic Evolution of Thermophiles

Environmental changes such as temperature shifts induce genomic evolution, which in turn provides the bacteria with thermal-tolerant abilities to survive under high temperatures. Such evolutionary changes could be achieved through horizontal gene transfer (HGT), gene loss, or gene mutations (4).

Horizontal Gene Transfer in Thermophiles

Horizontal gene transfer, which permits the exchange of DNA among organisms of different species, is an important driving force for bacterial adaptation and bacterial genome evolution (13, 28, 45, 46, 80). HGT occurs in two ways: either the new sequence replaces the homologous sequence or the sequence is acquired through gene integration by transduction, conjugation, and transformation. A comparison of hundreds of sequenced genomes demonstrates that >20% of the bacterial genes and >40% of the archaeal genomes are horizontally transferred (32, 62, 67, 81). In the thermophilic bacteria Thermotoga maritima and Aquifex aeolicus, ~24 and 16.2% of the genes were introduced from archaeal thermophiles by HGT (1, 60). Many
of the HGT-acquired genes bestow thermophilic traits that are essential for survival under extreme conditions. One prominent example is reverse gyrase, which is considered a thermo-adaptation trait that was transferred from archaea to bacteria (29, 34). Reverse gyrase is a DNA topoisomerase that introduces positive supercoiling to increase the melting temperature of double-stranded DNA. Once the reverse gyrase gene was deleted from the chromosome of *T. kodakarensis*, the mutant strain grew slowly under high temperatures, demonstrating the critical contribution of reverse gyrase to thermophily (3, 65, 81). The presence of thermophilic traits in a thermophilic organism’s genome that were transferred from other species indicates that HGT is effective in acquiring thermo-adaptive capacities. The contribution of horizontal gene transfer to the increased OGT in thermophiles was recently reviewed, and it was noted by Van Wolferen that thermophiles might not exist without gene exchanges among species (80).

**Gene Mutations in Thermophiles**

Since DNA is more unstable at higher temperatures, the DNA repair system should be more stringent in thermophiles to maintain genomic stability. A genomic analysis of mutations in thermophiles, such as *Thermus thermophilus* and *Sulfolobus acidocaldarius*, revealed that base substitutions occur at a lower frequency in thermophiles than in mesophiles (24), and a recent study confirmed these estimations (42). A hypothesis is thus prompted in which the resulted amino acid substitutions from gene mutations are more deleterious in a thermophile than in a non-thermophile. Generally, non-synonymous substitutions are more deleterious than synonymous substitutions; therefore, the frequency of non-synonymous substitutions in thermophiles should be reduced. Friedman et al. estimated the numbers of synonymous and non-synonymous nucleotide substitutions per site between 17,957 pairs of orthologous genes from 22 pairs of closely related species of archaea and bacteria (30) and found that the average ratio of non-synonymous to synonymous substitutions in thermophiles was significantly lower than that in non-thermophiles, indicating that the proteins of thermophilic prokaryotes were subjected to unusually stringent functional constraints. Noort et al. further confirmed this observation and found that the amino acid substitution of lysine to arginine is associated with thermophily (79).

However, some gene mutations may be beneficial to thermal adaptability. Blaby et al. (11) utilized a long-term culture apparatus, the Evolugator, to generate a thermophilic descendant from a mesophilic ancestor (*Escherichia coli* MG1655) and then whole-genome sequenced the sequentially isolated strains throughout the thermo-adaptation process. Several of the identified genetic alterations contributed to thermal tolerance, such as mutations in the *glpF* and *fabA* genes. Transforming the evolved thermo-tolerant strain (EVG1064) with a wild-type allele of *glpF* reduced fitness under high temperatures, whereas the mutation in *fabA* predictably increased the degree of saturation in membrane lipids, which is a known adaptation to elevated temperatures.

**Genome Reduction and Gene Loss in Thermophiles**

Prokaryotic genomes are compact and contain little inter-genic DNA compared with eukaryotes. This genomic feature benefits a short division time for rapid reproduction and also reduces the energy consumption for nucleotide synthesis. Intriguingly, the genome sizes of thermophiles are generally smaller than those of non-thermophiles, since all species that live at temperatures >60°C have genomes smaller than 4 Mb, whereas all species with genomes larger than 6 Mb live at temperatures lower than 45°C (79). As expected, the protein lengths and the number of protein family members in thermophiles are reduced compared with their homologous counterparts in non-thermophiles due to the small size of the genomes of thermophiles. A comparison of the genomes of *Thermus thermophilus* and *Deinococcus radiodurans* revealed systematical gene loss in *T. thermophilus*, including the urease complex, the ramnose metabolism pathway, acyl CoA:acetate/3-ketocid CoA transferase, fructose transport and utilization, and glycerol metabolism (61). Another similar example is *Thermoanaerobacter tengcongensis*, in which some of the TCA cycle-related genes are absent in the genome (5). These observations led to a hypothesis in which the reduction of functional complexity of a genome is likely a cost-minimizing mechanism for thermophiles to adapt to the environmental temperature (16, 23, 25, 70). However, whether thermophiles could eliminate those genes encoding proteins with low thermo-stabilities during evolution is still debated (37, 69).

**Base Biases of Thermophilic Genomes**

Genomic structure in thermophiles is thought to be more stable than that of mesophiles. Although the contents of guanine (G) and cytosine (C) in the genome are important indicators of DNA stability, large-scale genomic comparisons between thermophiles and mesophiles have been conducted to evaluate the nucleic acid compositional differences.
The GC content in some thermophiles is different from that of mesophiles, such as Thermus thermophilus ATCC 33923 with a GC content of 69.41% (38), Geobacillus kaustophilus with 52.1% (75), and Thermus sp. strain CCB_US3_UFI with 68.6% (76).Muston et al., therefore, hypothesized that a high GC content contributes to the thermostability of the genome and is correlated with the OGT (57, 58). Additionally, tRNAs and rRNAs, the translational machinery of some thermophilic organisms, were reported to have high GC contents as well (5, 6, 70, 83). Some investigators, however, have argued that some microbes have different OGTs but share similar and even lower GC contents, such as Caldicellulosiruptor hydrothermalis containing only 35% GC with an OGT of 70°C (12). Therefore, the GC composition seems to be independent of thermophily, at least not universal to all thermophiles (6, 70, 82, 90). On the other hand, a significantly high AG content in mRNAs is observed as a selective response for survival among thermophiles. Compared with mesophilic species, thermophilic mRNAs exhibited an enrichment of purines and purine clusters with significantly high purine/pyrimidine ratios, especially biased toward those genes encoding central elements of transcriptional and translational machinery, such as ribosomal protein and histone-like protein genes (7, 63). The correlation of the purine content and OGT, however, lacks further confirmation (52). Therefore, it is certain that the base bias contributes to thermophily, but the correlation between the base bias and thermophily should be evaluated using more factors, such as the growth environment and the Gram-positive or -negative features of the bacteria.

The pattern of synonymous codon usage in protein-coding sequences is another important feature in evaluating the genomic structure of thermophilic species. The synonymous codon usage in thermophiles is different from that of mesophilic species, mainly in arginine and isoleucine codons in which thermophiles more frequently use the AGG, ATA, and AGA codons and avoid CGT and CGA (36, 49, 51, 74). The exhaustive genomic evaluation of nucleotide combinations suggests that A and G appear as nearest neighbors with a high frequency in thermophile genomes (90). Basak et al. postulated the bias usage of codons possibly resulted from the maintenance of codon-anticodon interaction energy at an intermediate strength so that the translation process could proceed smoothly (8). A recent study revealed that the synonymous codon usages are related directly with the OGT of the encoded enzyme activity (48). The differences in synonymous codon usage in thermophiles are thus believed to be important factors that are strongly linked with the selective pressure of the growth temperature.

Obviously, the base biases directly impact the variations of amino acid usages in proteins. The analyses of the proteins of thermophilic organisms suggest that the amino acid composition in the proteomes of thermophiles is distinguishable from that of mesophiles. Although debating some observations is generally accepted, such as an increase in the frequency of charged residues (Glu, Arg, and Lys), a decrease in the frequency of polar uncharged residues (Asn, Gln, Ser, and Thr), a decrease in the frequency of thermo-labile amino acids (His, Gln, and Thr), and an increase in the (Glu + Lys)/(Gln + His) ratio in thermophiles (27, 43, 74, 77). In addition, Zeldovich et al. claimed that the amino acid sequence IVYWREL might serve as a universal proteomic signature of thermophilic features for prokaryotic microorganisms (90). The special amino acid usage, therefore, is reasoned as a strategy for thermo-adaptation.

### The Global Gene Expression Responses in Thermophiles to High Temperatures

Generally, thermophiles can survive relatively wide ranges of temperature, indicating that thermophiles can elicit a prompt physiological response to changes of environmental temperature and form a functional network within cells by maintaining the optimal expression status of certain genes. The transcriptome and proteome data at the opposite sides of central dogma represent the gene expression status in thermophiles. Moreover, the integration of the two sets of gene expression data forms a solid foundation to pursue the adaptive mechanisms of thermophiles at high temperatures.

### Temperature Response of Operon Genes in Thermophiles

The assembly of genes into operons is viewed as an important adaptation process of microbes to environmental changes, and approximately half of the genes in those species are located in operon structures. Euryarchaeotal methanogens have the highest density of operons, on average 60%, whereas thermophiles have ~43–56%, suggesting that the degree of operonization in different species is not correlated with the OGT. The operon structure, however, is believed to be more stable in thermophiles than in non-thermophiles (88). More importantly, the expression statuses of operon genes are correlated with thermophily. For instance, the analysis of Thermoanaerobacter tengcongensis through a TransOmics strategy revealed that the expression of ~250 genes was temperature-dependent and that most of the differential genes at the mRNA
level were correlated strongly with the protein level. Intriguingly, an analysis of the genomic locations of these temperature-dependent genes revealed that >30% of these genes were in contiguous units with relevant biological functions. A theoretical prediction suggested that these gene clusters are potential operons (20). Transcriptomic and proteomic studies of another thermophiles, *Thermotoga maritima*, revealed that ~15 predicted gene operons were differentially expressed under high temperatures, including the most well known heat-shock operons *hrcA-grpE-dnaJ* (TM0851-TM0850-TM0849), *groES-groEL* (TM0505-TM0506), and *dnaK-sHSP* (TM0373-TM0374) (66).

A large body of evidence regarding gene expression in thermophiles indicates that operon regulation is an important mode to retain thermophile survival, because the genes located within an operon could be economically co-regulated responding to a stimulus. Moreover, these co-expressed operons in thermophiles are possibly co-regulated through global regulators, which is an efficient way for the micro-organism to promptly respond to environmental changes. A question naturally prompted then is whether there are some regulators that globally control the expression of gene clusters responding to environmental temperature changes. Some global regulators, such as CRP, IHF, FNR, FIS, ArcA, Lrp, and H-NS, have been reported in *E. coli*, which corresponds to the transcriptional regulation of >50% of *E. coli* genes (15). The global regulatory systems sometimes overlap and can cross-talk, such as σ32, a heat-shock transcription factor, regulating not only the expression of heat-shock response proteins but also the chaperon proteins. On the other side, in some cases, the response of genes to different stresses are common, not only to heat but also to other conditions (86). Therefore, it is possible to deduce that there are global regulator systems in thermophiles, which may respond not only to temperature but also to other stresses by regulating the expression of gene clusters.

**The Stable and Efficient Protein Synthetic Machinery in Thermophiles**

Since maintaining a sufficient number of proteins is necessary for functional performance, the protein synthetic machinery in thermophiles is expected to be stable and efficient under high temperatures. The central elements of translational machinery include mainly tRNA, rRNA, and ribosomal proteins. As previously mentioned, the thermophile tRNA and rRNA with high GC contents are more stable than those of mesophiles. What about the protein components of the machinery? The proteomics analysis of *Thermoanaerobacter tengcongensis* suggested that the abundance of ribosomal protein S1 was significantly upregulated under higher temperatures than under the OGT (20). The proteomics study regarding a thermophilic bacterium *Bacillus methanolicus* MGA3 revealed the abundant increase of several ribosomal protein members, such as ribosomal protein L17, 30S ribosomal protein L14, and 30S ribosomal protein S18, in addition to the ribosomal-associated protein ribosome-binding factor A when the bacterium grow at higher temperature than the OGT (56). Another study integrated of proteome and transcriptome on *Pyrococcus furiosus* also found that ribosomal proteins, such as the LSU ribosomal proteins L10E, L12A, and L7AE, had obviously higher abundances at 90°C than at 70°C (78). It has been reported in *E. coli* that the deletion of rbfA triggers cold shock response and lower protein synthesis (40). A logical deduction regarding these analyses thus is that the upregulated ribosomal proteins are required for efficient protein synthesis against heat stress in thermophiles. In addition, both archaeal and bacterial ribosomal protein complexes in thermophiles have a higher affinity to 23S rRNA than do their mesophilic counterparts, whereas their structures are more compact (68, 71, 87). These findings strongly indicate that the translational machinery of thermophiles is so stable and efficient that functional proteins can be synthesized at sufficient amounts.

**The Expression of Temperature-Sensitive Genes in Thermophiles**

Increasing evidence indicates that some gene expression products play critical roles in the thermal adaptation of thermophiles. These genes are generally considered to possess specific functions under higher temperatures and are upregulated at either the transcriptomic or proteomic level in response to temperature changes.

Heat shock proteins (HSPs) are the best known proteins that respond to heat stress and protect against cellular damage induced by heat stress (55). The upregulation of HSP genes in thermophiles has been widely observed at both the transcriptional and proteomics levels. For instance, at the transcriptional level, *Pyrococcus furiosus* triggered the upregulation of *Hsp60* and *Hsp20* during a temperature shift from 90°C to 105°C (73); the expression of *groEL-grovES, hrcA-grpE-dnaJ*, and *dnaK-sHSP* in *Thermotoga maritima* was induced in response to a temperature increase from 80°C to 90°C (39), whereas in *Sulfolobus solfataricus* sHSPs were upregulated in response to a temperature shift from 80°C to 90°C in 5 min. At the proteomic level, the upregulation of chaperonin proteins, such as GroEL, GroES, DnaK, and GrpE, in response to a...
temperature increase was observed in several thermophilic species, including *Thermoanaerobacter tengcongensis* and *Thermotoga maritime* (19, 84, 85). A proteomic analysis of *Thermoanaerobacter tengcongensis* further revealed that heat shock proteins, such as HSP60 (TTE0580), sHSP (TTE2587), and HSP10 (TT0579), interact widely with other proteins to form complexes (54), indicating a protection role of HSPs to other proteins.

Glycolysis pathway-related proteins are thought to be key elements that are associated with thermophily by providing immediate energy for thermophiles to cope with heat stress. Therefore, the expression status of these proteins in thermophiles reflects the physiological response. A proteomic analysis of *Geobacillus* sp. NTU 03 under a rapid temperature increase showed that seven dehydrogenases utilizing NADH as electron donor were downregulated (72), and a quantitative proteomics study of *Thermoanaerobacter tengcongensis* (19, 21) revealed that the genes that are involved in aerobic respiration or sulfur respiration, such as the genes of NADH dehydrogenase and electron transfer flavor protein, were also downregulated. In contrast to the attenuation of NADH-dependent enzymes and respiratory-related genes, the genes of the glycolysis pathway, such as GAPDH, PGK, and PGL, were globally upregulated at the mRNA and protein levels in response to increasing temperatures in several thermophiles, including *Thermus thermophilus*, *Thermoanaerobacter tengcongensis*, *Thermotoga maritima*, and *Geobacillus thermoglucosidasius* (21, 31, 44, 50, 85). In addition, glycolysis was upregulated, and the TCA cycle was downregulated in response to an environmental switch from a high redox state to a low redox state in *Geobacillus thermoglucosidasius* (50); it could be deduced accordingly that the redox state might be attenuated under heat response. A model was proposed and is illustrated in FIGURE 1 regarding the energy generation processes in *Thermoanaerobacter tengcongensis* in response to temperature change. During environmental temperature increase, the transcriptional regulators of the gene clusters of sulfur respiration and glycolysis are activated, and an inverse coordination network

![FIGURE 1. A schematic diagram illustrating the energy-generation process in T. tengcongensis during thermal responses](https://physiologyonline.org/)

In the rectangular box are the proteins that are upregulated in response to an increase of the growth temperature, which are mainly located in the cytoplasm and are involved in glycolysis and the related pathways. In the elliptical box are the proteins that are downregulated in response to an increase in the temperature, which are dominantly located on or associated with the membrane and are involved in the sulfur-respiration-related system. NitroR, nitroreductase; Ndh, NADH dehydrogenase FAD-containing subunit; Nuo EFG, NADH dehydrogenase/NADH:ubiquinone oxidoreductase subunit E/F/G; Etf, electron transfer flavoprotein; PFOR, pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin oxidoreductase; Fe-S Cluster, Fe-S-cluster-containing hydrogenase; BCKDHC, branched-chain alpha-keto acid dehydrogenase complex; FNR, ferredoxin-NADP(H) reductase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; OADC, oxaloacetate decarboxylase; PDC, pyruvate dehydrogenase complex.
in gene expression is formed, leading to the attenuation of the respiration capacity of the membrane proteins and an increase in the energy generation through the carbohydrate metabolism (19).

The expression of other proteins groups, such as antioxidant proteins and VapBC proteins, responds to temperature shifts as well. With an increase in the environmental temperature, some antioxidant proteins in thermophiles, such as thioredoxin peroxidase, rubredoxin, and SOD (44, 78), were upregulated, indicating that the temperature increase may be accompanied by oxidative stress. However, the VapBC proteins, the largest family of type II antitoxin system and the virulence-associated proteins in prokaryotes, are triggered by temperature shifts, as revealed by a global transcriptomic analysis of Sulfolobus solfataricus. The knock out of a vapBC locus in S. solfataricus substantially changed the transcriptomic profile and rendered these archa heat shock labile (22).

Thermostability of Proteins in Thermophiles

Unlike pH or salt, temperature impacts cells with no difference between the outer and inner cellular boundaries. One challenge for thermophiles grown under high temperatures is to stabilize the cellular proteins in their native configurations. How the thermophilic species maintain protein stability under such high temperatures has attracted attention for a long time but is limited to the status of individual proteins. Recent proteomic developments offer more convincing evidence supporting protein stability in thermophiles.

The Functional Categories of Thermostable Proteins

It is believed that not all of the proteins in thermophiles are thermally stable. An adaptation through the increase in temperature appears to be concentrated in the proteins with catalytic activities as well as in regulator proteins (32). Yun et al. identified a subset of hyperthermostable proteins in the cytosolic proteome after heat treatment in vitro. The identification list suggested that those proteins were function-required and heat-resistant in thermophiles and included intracellular protease, thioredoxin reductase, triosephosphate isomerase, hydroperoxide reductase, proteasome-related proteins, and translation initiation factors (89). Furthermore, a proteomic analysis of thermophiles demonstrated that the number of detectable proteins at high temperature is obviously lower than that at the OGT, additionally indicating that the thermostable proteins are concentrated in certain functional categories that should be necessary for thermophile survival under high temperatures (44, 84, 85).

More Disulfide Bonds in Thermophilic Proteins

Using genomic calculations, Mallick et al. (53) found that some thermophiles have a higher number of disulfide bridges than do mesophiles, and it was proposed that certain thermophiles use disulfide bonding as a major mechanism for protein stabilization (9, 41, 53). A subsequent proteomic analysis of thermophiles validated this postulation (14, 35). For example, the quantitation of disulfide bonds by fluorescence labeling in Pyrobaculum aerophilum revealed that ~47% of the cysteine residues in the cell lysate were involved in the formation of disulfide bonds, whereas only ~8% of these residues were found in E. coli. Moreover, through two-dimensional diagonal gel electrophoresis (2D-DGE), the frequency of protein complexes that were formed by disulfide binding in Pyrobaculum aerophilum was significantly higher than that in E. coli. The structures regarding proteins in various hyperthermophilic archa also support the widespread disulfide bonds in thermophiles (17, 47). Since thermophilic proteins and their complexes tend toward compact structures, the formation of disulfide bonds supposedly favors this process (14).

The use of disulfide bonding is surprising, however, because the intracellular environment is generally in a reducing status, preventing the formation of disulfide bonds. Therefore, it becomes a question about the intracellular...
The Correlation of Protein Thermostability With Protein Complexes

It is generally accepted that proteins are not independent units within a cell but interact with each other to form an intricate network that enables an effective communication among these molecules. Approximately 20% of the proteins in thermophiles are predicted to be involved in the formation of complexes, including homomorphic complexes, heteromeric complexes, and supercomplexes (26). An analysis of *Thermoanaerobacter tengcongensis* revealed that numbers of protein complexes could be separated by blue native electrophoresis, in which some complex components were identified (54). Moreover, these complex components were variable due to changes in the culture temperature, indicating that the protein interactions in thermophiles are temperature-dependent. For instance, the number of HSPs in the complexes increased as the temperature increased, indicating that the HSPs further protected those interacting proteins under higher temperatures. This hypothesis was confirmed by the study of thermal stability of glucokinases (GLK) in *Thermoanaerobacter tengcongensis*. There are two types of GLK in this bacterium: ADP-dependent (ADP-GLK) and ATP-dependent (ATP-GLK). ADP-GLK are quite stable and active at 80°C and are strongly associated with HSP60, whereas ATP-GLK are unstable and inactive at 80°C due to a lack of interaction with HSP60 (67).

Summary and Perspectives

The heat shock responses and thermal acclimations are ubiquitous mechanisms in thermophiles, which represent an interesting model to explore the key molecular events protecting an organism from heat stress. As summarized in **FIGURE 2**, from an omics perspective, the adaptation of thermophiles to high temperatures is a combination of different strategies, including genetic selection and functional acclimatization. Genomics studies of thermophiles have demonstrated that the evolution of thermal tolerance depends on the levels of heritable variations, such as genomic size shrinkage, horizontal DNA transfer, and gene mutation. Although the analyses of gene expression in the transcriptome and proteome in thermophiles illustrate that the global transcriptional regulation of certain operons containing key functional genes is an efficient response to temperature increases, supported by the thermostable and efficient protein synthesis machinery. The formation of a functional network is critical as well for thermophilic acclimatization, especially for those proteins functioning under high temperatures. Proteomics evidence has demonstrated that most thermophiles contain gene clusters that encode proteins with high thermostability and have pivotal functions for living cells, including proteins in the HSP family, glycolysis pathway, antioxidants, and antitoxins. Impressively, protein interactions are also involved in controlling thermal tolerance. The omics data of thermophiles therefore deliver a clear and overall view of the macromolecules participating in heat shock responses.

The omics data of the discovery of temperature-dependent macromolecules in thermophiles is just a step toward the exploration of the thermal adaptation mechanism. Many questions remain to be addressed through the tight integration of the omics approaches. First, the correlation of thermo-adaptation and genomic changes, such as gene gains and genomic size changes, has not been verified at the functional level due to a lack of the evidence of gene expression during either transcription or translation. Second, a recent analysis of DNA methylation and micro-RNAs has been used to investigate the bacterial genome; however, how these elements contribute to genomic thermostability in thermophiles has not yet been reported. Third, the proteins that are bound to chromosomes, such as histones, can significantly impact the genomic DNA stability, whereas the relationship between the chromosome proteins and genomic DNA in thermophiles has very limited information. Fourth, since the heat shock response to high temperature is a dynamic process that is both temperature- and time-dependent, dynamic data of gene expression are necessary for the establishment of a thermo-adaptive model. Current investigations of transcriptomics and proteomics, however, do not examine the abundance of changes in gene expression over time courses. Fifth, the relationship of metabolomics and adaptation of thermophiles still lacks study. As is known, some thermophiles are industrially valuable because they secrete kinds of bioenzymes and biofuels. It should be recognized that the process of enzyme production and carbohydrate degradation is possibly the process of environmental adaptation. All of these challenges should attract future studies of thermophiles using omics strategies.
This work was supported by a grant from the 973 program (2010CB912703), the Nature Science Foundations of China (30800023), and the Beijing Municipal Natural Science Foundation (5132023).

No conflicts of interest, financial or otherwise, are declared by the author(s).

Author contributions: Q.W., Z.C., and J.Z. drafted manuscript; Q.W. edited and revised manuscript; Q.W. approved final version of manuscript; Z.C. prepared figures.

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