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Replication initiator DnaA interacts with an anti-terminator NusG in *T. tengcongensis*

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ABSTRACT

DnaA plays a central role in initiation of DNA replication at *oriC* in bacteria, and is also a transcription regulator which interacts with the DnaA box relative to a specific gene. Through screening the interaction between TtDnaA and the transcription machinery in *Thermoanaerobacter tengcongensis* by yeast two-hybrid assays, we found for the first time that the TtDnaA could interact with an anti-terminator, TtNusG2, in this thermophilic bacterium. The direct interaction between TtDnaA and TtNusG2 was verified by surface plasmon resonance (SPR) assay *in vitro*, and was further confirmed by co-immunoprecipitation assay *in vivo*. Moreover, we demonstrated that domain I and domain III of TtDnaA were responsible for the interaction with TtNusG2. These findings might expand our understanding of cooperation of two fundamental processes, replication and transcription, in this bacterium.

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DnaA is virtually ubiquitous in the eubacterial world and is critical important in the initiation of DNA replication. It is structurally and functionally similar to origin-recognition complex (ORC) proteins from eukaryotes and archaea [1–3]. DnaA also plays an important role in the regulation of the timing of chromosome replication initiation during the cell cycle. This is achieved via an autoregulatory feedback loop in *Escherichia coli*, where DnaA binds to DnaA boxes downstream of the DnaA promoter [4]. During the initiation of chromosome replication in *E. coli*, DnaA not only interacts with DnaA itself but also with other replisomal proteins to fulfil its function, such as DnaB, and the clamp/clamp loader-complex of polymerase III in cooperation with Hda protein [5]. In addition to the primary function as the replication initiator, DnaA is also a sequence-specific transcription factor. It can be a transcription repressor, activator or terminator upon the location of DnaA box relative to promoters [6].

N-utilization factor G (NusG), an essential protein in *E. coli*, is widespread in bacterial and archaeal genomes [7], and plays multiple roles in transcription and translation. It participates in a variety of cellular and viral termination and anti-termination processes [8–12]. NusG is able to improve the efficiency of Rho-mediated transcriptional termination [11,13]; At the same time, NusG is an anti-terminator, it accelerates the rate of RNA chain growth by RNA polymerase (RNAP) [14], and makes RNA polymerase read-through transcription terminators in *rrn* operons [15,16]. Furthermore, NusG has been suggested to play a role in translation due to the presence of the KOW motif shared with ribosomal protein (r-protein) families [17]. Nevertheless, there has been so far no evidence that NusG takes part in DNA replication.

Thermoanaerobacter tengcongensis is an anaerobic, rod shaped and low-GC (33%) thermophilic eubacterium, which was isolated from a freshwater hot spring in China and grows well at 75 °C [18]. The genome sequence of *T. tengcongensis* has been determined [19], and the TtDnaA involved in replication initiation in this thermopile has been comprehensively investigated recently [20]. This paved the way to explore the functions of specific genetic factors and the interactions between them. In this study, we demonstrate that TtDnaA and one of TtNusGs from *T. tengcongensis* interacts with each other *in vitro* and *in vivo*, thus suggesting for the first time that NusG might be involved in the process of DNA replication.

Materials and methods

Bacteria, plasmids and oligonucleotides. Escherichia coli DH5α was used as a host for the cloning experiments, and *E. coli* BL21DE3 (lysS) for overproduction of the recombinant proteins. *T. tengcong-*

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Table 1
Plasmids used in this study

Plasmids	Description	Sources or references
pET-28a	Kan^r, expression vector with His-tag coding sequence	Novagen
pET-23b	Amp^r, expression vector with His-tag coding sequence	Novagen
p28TtDnaA	pET-28a derivative for expression of the TtDnaA	20
p23TtNusG2	pET-23b derivative for expression of the TtNusG2	This work
pGBKT7(BD)	Yeast two-hybrid DNA-binding domain vector	Clontech
pGADT7(AD)	Yeast two-hybrid activation domain vector	Clontech
BD-TtDnaA	pGBKT7 derivative for expression of the TtDnaA	20
AD-TtDnaA	pGADT7 derivative for expression of the TtDnaA	20
BD-TtNusG2	pGBKT7 derivative for expression of the TtNusG2	This work
AD-TtNusG1	pGADT7 derivative for expression of the TtNusG1	This work
AD-TtNusG2	pGADT7 derivative for expression of the TtNusG2	This work
AD-TtNusG3	pGADT7 derivative for expression of the TtNusG3	This work
BD-TtDnaA1	pGBKT7 derivative for expression of the TtDnaA region 1	This work
BD-TtDnaA2	pGBKT7 derivative for expression of the TtDnaA region 2	This work
BD-TtDnaA3	pGBKT7 derivative for expression of the TtDnaA region 3	This work
BD-TtDnaA4	pGBKT7 derivative for expression of the TtDnaA region 4	This work

Table 2
Oligonucleotides used in this study

Names	Sequences(5′ to 3′)[a]	Purposes
P1	ACGGATCCTGAGTGAAGTACAAGAGTT	P1/P2: pGADT7-TtNusG2,
P2	TCCTCGAGCTGCAGAAAAATCTATCCCCATCTC	pGBKT7-TtNusG2
P3	TAGGATCCACCGTCAAATTTGGGAGA	P3/P4: pGBKT7-TtDnaA1
P4	AACTGCAGTACGCTTTTGCTGGAGAT	
P5	ATGGATCCACAGAGAGATAAAAGAGTC	P5/P6: pGBKT7-TtDnaA2
P6	ATCTGCAGTCCGGGATATTGAGGTTT	
P7	TAGGATCCCTCCAGCAAAAGCGTACA	P7/P8: pGBKT7-TtDnaA3
P8	ATCTGCAGTGAGCTTTACTGTTATTTC	
P9	ATGGATCCTAGCTTATGTTGCAGAAAAA	P9/P10: pGBKT7-TtDnaA4
P10	AACTGCAGAACCTTTTATCCTTTTTTTA	
P11	ACGGATCCTATGAGTGAAGTACAAGAGT	P11/P12: p23TtNusG2
P12	TCAAGCTTTTGTATTTTCATGATCTG	
P13	CGGAATTCAAGTGGTATGTGCTGTTTA	P13/p14: pGADT7-TtNusG1
P14	AGGGATCCTTTTCTCAACAATTTCAAGC	
P15	TTGAATTCTCTGAAAAGGAAACTGCG	P15/p16: pGADT7-TtNusG3
P16	TTGGATCCTTTTCTGAAGTTGCACAAG	
P17	TACATATGATGTATGGTGATTACCGTC	P17/18: pGADT7-TtDnaA,
P18	TCGGATCCACCTTTTATCCTTTTTTTAAG	pGBKT7-TtDnaA

[a] Restriction sites are underlined.

ensis was grown in the modified MB medium at 75 °C without shaking [18,19]. The plasmids and oligonucleotides used in this study are described in Tables 1 and 2, respectively.

Yeast two-hybrid assays. Yeast two-hybrid analysis was carried out using the Matchmaker system 3 (Clontech, Palo Alto, CA, USA) according to the manufacturer's protocol. Genes encoding TtDnaA (TTE0001) and TtNusGs (TtNusG1, TTE0730; TtNusG2, TTE2111; TtNusG3, TTE2307) were amplified from the *T. tengcongensis* genomic DNA by PCR (for primer sequences, see Table 2). The PCR products were digested with appropriate restriction enzymes and cloned into both pGADT7 and pGBKT7 to generate the AD (active domain) and BD (binding domain) fusion plasmids, respectively. Protein–protein interactions were investigated by co-transformation of yeast AH109 with respective pairs of AD and BD fusion plasmids. The co-transformants were streaked on SD-Leu/-Trp plate and cultured at 30 °C for 24 h. Cells were then resuspended in sterile TE (10 mM Tris–Cl, 1 mM EDTA, pH 8.0), and 5 μl of the cell suspension were spotted on SD-His/-Leu/-Trp + 5 mM 3-AT plate, and SD-His/-Leu/-Trp/-Ade/X-α-gal plate. The plates were incubated at 30 °C for 3 days.

Cloning, expressing and purification. The TtNusG2 gene of *T. tengcongensis* was amplified with primers P11/P12 (Table 2) from genomic DNA by PCR and inserted into the BamHI/HindIII sites of the expression vector pET23b (Novagen) to generate the expression plasmid p23TtNusG2. *E. coli* BL21DE3(lysS) harboring the p23TtNusG2 was used to overproduce the His-tagged TtNusG2 protein by induction with 0.5 mM IPTG for 3 h. The cells were pelleted and resuspended in buffer A (0.3 M NaCl, 10 mM imidazole, 50 mM sodium dihydrogen phosphate) and lysed by ultrasonication. The cell lysates were centrifuged at 12000 rpm for 30 min and the clarified supernatants were loaded onto the Ni–NTA (Ni^{2+}–nitrilotriacetate)–agarose column (Novagen) according to the manufacturer's instructions. The purified TtNusG2 proteins were desalted by Amicon desalt column using buffer B (10 mM Tris–HCl pH 7.0, 2 mM DTT). TtDnaA was expressed and purified as previously described [20]. Protein concentrations were determined by using the BCA™ protein assay kit (PIERCE).

Surface plasmon resonance (SPR) assay. Interaction of purified TtNusG2 and TtDnaA proteins was analyzed on a BIAcore™ 3000 instrument (BIAcore AB, Uppsala, Sweden) as described previously [21], with minor modifications. All reagents except fusion proteins were purchased in prefiltered and degassed form from BIAcore, and the SPR assays were performed at 25 °C using 0.01 M HEPES, 0.15 M NaCl (pH 7.4) and 3 mM EDTA as running buffer. The TtNusG2 was immobilized on a CM5 sensor chip (BIAcore AB) at 3500 RUs by amine coupling according to BIAcore protocol. The purified protein TtDnaA was diluted in running buffer at a concentration 20 μg/ml, and injected at a flow rate of 20 μl/min for 3 min, and then running buffer was allowed to flow over the surface for 5 min for dissociation data. For a negative control, the purified pro-

tein GST at concentration 20 μg/ml was injected with the same manner. The flow cells were regenerated by a single 30 s pulse with 10 mM glycine–HCl (pH 1.5).

Co-immunoprecipitation and immunoblotting. Polyclonal antibodies against TtDnaA and TtNusG2 were prepared respectively by immunizing BALB/c mice with purified recombinant proteins. To determine the interaction between TtDnaA and TtNusG2 *in vivo*, *T. tengcongensis* cells from overnight cultures were collected and resuspended in 40 mM Tris–HCl, pH 8.5, and 10 mM DTT, followed by ultrasonication and the lysates were centrifuged at 12000 rpm for 30 min at 4 °C. The supernatant was subjected to immunoprecipitation with corresponding antibodies and the protein A beads (Clontech). Proteins present on the protein A beads were released by boiling in the SDS loading buffer and detected by immunoblotting with anti-TtDnaA or anti-TtNusG2 antibodies.

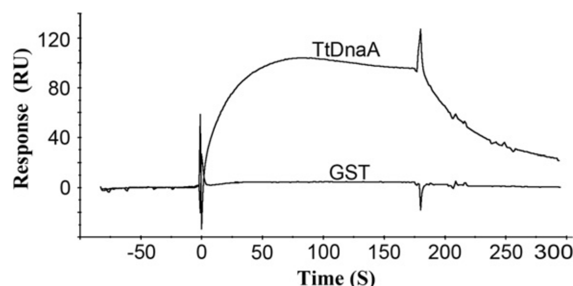

Fig. 2. Surface plasmon resonance (SPR) analysis of the interaction between TtDnaA and TtNusG2. The binding of TtDnaA and negative control GST to immobilized TtNusG2 was shown. Data were obtained by running a concentration of 20 μg/ml TtDnaA or GST, respectively, over the surface of a CM5 sensor chip immobilized with TtNusG2.

Results

Identification of TtNusG2 as a potential TtDnaA interacting protein by yeast two-hybrid system

Due to the important role of DnaA in DNA replication and its multiple functions in gene expression regulation, yeast two-hybrid assay was employed to screen the TtDnaA-interacting proteins from the transcription machinery of *T. tengcongensis*. One of the potential TtDnaA interactors was found to be TtNusG2, which was evidenced by yeast two-hybrid assays. As shown in Fig. 1, co-transformants containing either BD-TtDnaA/AD-TtNusG2 or BD-TtNusG2/AD-TtDnaA could grow on the selective media, and activate the lacZ reporter gene in these colonies with color in blue, just as the positive control provided by the manufacture. Interestingly, this interaction is quite specific, as no such an interaction between TtDnaA and the other two putative TtNusG proteins was observed in *T. tengcongensis*. Moreover, no growth was observed for the yeast cells co-transformed with pGBKT7-laminC/AD-TtNusG2 (negative control), pGADT7/BD-TtNusG2, or pGADT7/BD-TtDnaA plasmids. Similarly, co-transformants harbouring both pGBKT7/AD-TtNusG2 or pGBKT7/AD-TtDnaA were also not able to grow on selective media, which means neither AD-TtNusG2 nor AD-TtDnaA could activate the expression of the reporter gene by themselves (Fig. 1). These results suggest that the TtNusG2 is a potential TtDnaA-interacting protein.

TtDnaA binds to TtNusG2 in vitro

To verify that the interaction of TtNusG2 and TtDnaA revealed by the yeast two-hybrid assays was due to a direct interaction of these two proteins, Surface plasmon resonance (SPR) assay was performed to detect the interaction *in vitro*. Both TtNusG2 and TtDnaA were expressed and purified from *E. coli* BL21DE3 (lysS). As shown in Fig. 2, the signal of TtDnaA binding to TtNusG2 was significant in the SPR analysis with a response value of 100 RU, consistent with the results from yeast two-hybrid assays. In order to confirm this interaction is specific, the unrelated protein GST was used as a negative control, and there was no any detectable binding response when purified GST protein was passed over the chip immobilized with TtNusG2 (Fig. 2). These results confirm that the TtDnaA directly and specifically binds to TtNusG2 *in vitro*.

TtDnaA interacts with TtNusG2 in vivo

To investigate if TtDnaA indeed interacts with TtNusG2 *in vivo* in *T. tengcongensis*, then we examined the interaction between TtDnaA and TtNusG2 by co-immunoprecipitation experiments. The whole cell lysates were immunoprecipitated with anti-TtDnaA or anti-TtNusG2 antibodies respectively, followed by pull-down with the protein A beads. Taken samples omitting antibodies as the negative control, the immunoprecipitated complexes were separated on SDS–PAGE, and analyzed by Western blotting with anti-TtNusG2 or anti-TtDnaA polyclonal antibodies, respectively. As expected, the TtDnaA or TtNusG2 was clearly detected when precipitated with anti-TtNusG2 or anti-TtDnaA antibodies respectively (Fig. 3). The TtDnaA likely expressed in a relatively low level at period of life cycle when we collected the samples for western blotting, as only a weak signal of TtDnaA was observed in cell extract (Fig. 3A). However, it was still significantly pulled down by TtNusG2 in this immunoprecipitation assay. Thus, these data confirm that TtDnaA interacts with TtNusG2 effectively in the *T. tengcongensis* cells.

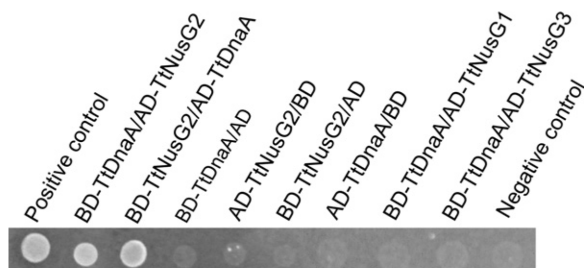

Fig. 1. Yeast two-hybrid analysis of interaction between TtDnaA and TtNusG2 from *T. Tengcongensis.* Constructs encoding AD and BD fusions of TtDnaA and TtNusG1-3 (Table 1), were co-transformed into yeast AH109. The transformants were grown and spotted on SD-His/-Leu/-Trp/-Ade + 5 mM 3-AT media in the presence of X-α-gal. The colonies could grow and turn blue ($LacZ^+$), indicating interaction in yeast cells. Both positive and negative controls were set up as described by the manufacturer.

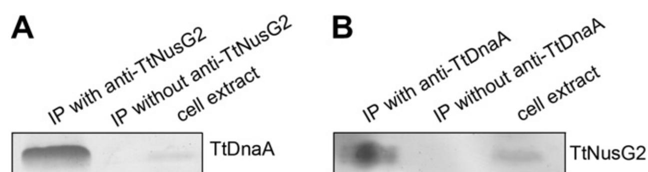

Fig. 3. Co-immunoprecipitation analysis of the interaction between native TtDnaA and TtNusG2 *in vivo*. The extracts of the growing *T. Tengcongensis* cells were incubated with antibodies against TtNusG2 (A) or TtDnaA (B), and precipitated with protein A beads. Immunocomplexes were released from the beads by boiling, and proteins in the immunocomplexes were identified by immunoblotting with anti-TtDnaA (A) or anti-TtNusG2 (B) antibodies, respectively. Total cellular extracts and the precipitated samples without corresponding antibodies were loaded as positive and negative controls.

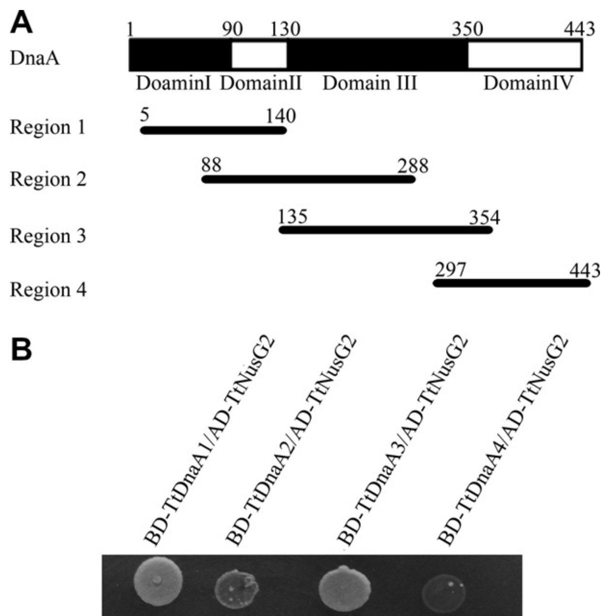

Fig. 4. Yeast two-hybrid analysis of the interactions between TtNusG2 and various regions of TtDnaA. (A) Schematic representation of the full length of TtDnaA and four functional domains [20]. The corresponding regions 1–4 were constructed in the pGBKT7 vector. The number indicated amino acid residues. (B) The BD fusion Constructs (BD-TtDnaA1 to 4) encoding the regions 1 to 4 of TtDnaA were co-transformed with AD-TtNusG2 into the yeast AH109. The transformants were analyzed as described in Fig. 1.

Determination of the TtDnaA regions required for the interaction with TtNusG2

According to the DnaA from *E. coli* [22,23], TtDnaA has been divided into four functional domains [20]. Here, we investigated which region of TtDnaA was involved in the interaction with TtNusG2. Constructs expressing the BD fusions of four regions overlapping the domain I to IV of TtDnaA were prepared respectively (Fig. 4A), and were subjected to check the interactions with TtNusG2/AD fusion by yeast two-hybrid assays. We found that TtNusG2 interacted strongly with Region 1 and Region 3, and very weakly with Region 2 (Fig. 4B). The weak interaction with Region 2 might be due to the partial overlapping with Region 1 and 3 in this construct, and no interaction was detected between TtNusG2 and the Region 4. Hence, it is likely that TtDnaA interacts with TtNusG2 through the domain I and III. We have also subdivided TtNusG2 into two regions based on the crystal structure of *Aquifex aeolicus* NusG (aaeNusG) [24], and prepared the corresponding constructs for yeast two-hybrid assays. But we did not observe any interaction of TtDnaA with these two separate regions (data not shown), which implies that TtNusG2 interacts with TtDnaA as a whole protein.

Discussion

In the present study, we identified that TtNusG2 could efficiently interact with TtDnaA both *in vitro* and *in vivo*, and the domain I and III of TtDnaA are likely responsible for this interaction. This is the first time that a link between NusG and DnaA has been reported.

It is interesting to find out that only TtNusG2 can interact with TtDnaA among three TtNusG proteins in *T. tengcongensis*. We have also tested the interactions between DnaA and NusG from *E. coli* and *B. subtilis* respectively, but no such an interaction was observed (data not shown). It was evidenced that the function of NusG is not completely conserved among bacteria, For example, NusG in *B. subtilis* is not as essential as in *E. coli* for viability or ρ-dependent terminator [11,25]. Among those microorganisms whose genomes have been completely sequenced, most of them just have one *nusG* gene, but some others have two or three *nusG* genes as *T. tengcongensis*. This implies that the function of NusG might have differentiated during the process of evolution.

A homology structure model for NusG of *E. coli* (ecoNusG) was created based on the crystal structure of aaeNusG [24]. Analyses suggest that ecoNusG and TtNusG2 just share two domains with aaeNusG, they are homologous to domain I and domain III of aaeNusG, respectively. Domain I similar to ribonucleoprotein (RNP) motif, and domain III homologous to r-protein L-24 encompassing a KOW motif [24]. Sequence analysis revealed that the counterparts of aaeNusG domain I and III are both conserved in all three TtNusGs, which means those unconserved region might determine the differences of these TtNusGs in interaction with TtDnaA. In our results, it was shown that TtNusG2 interacted with TtDnaA as a whole protein, thus we can deduce that the unconserved region might affect the protein conformation and lead to a divergent function among them.

It is noteworthy that the domain I and III, but not the DNA-binding domain IV of TtDnaA likely particpated in the interaction with TtNusG2. Since domain I of DnaA is involved in DnaA oligomerization and retention of DnaB in the *oriC*-prepriming complex, and domain III is responsible for ATP binding and hydrolysis [22], it is possible that at the period when TtDnaA binds to the *oriC*, the interaction between TtNusG2 and TtDnaA might modulate the replication initiation by affecting the TtDnaA oligomerization or its ATP dynamics.

Moreover, NusG interacts directly with both RNAP and Rho factor when enhancing ρ-dependent termination or increasing the read-through of RNAP [26–29]. Being a transcription factor, DnaA is also believed to interact physically with RNAP which was supported by rpoB mutants assay [30]. Therefore, it is reasonable to suppose that TtNusG2 might take part in the TtDnaA-mediated gene regulation. Notably, it has been shown that DnaA controls the frequency of DNA replication initiation by autoregulated expression [31–34]. When DnaA accumulates to a certain level in cell, the initiation is triggered [1,35]. Hence it is possible that TtNusG2 participates in the regulation of the timing of chromosome replication initiation by regulating the expression of TtDnaA, through interaction with TtDnaA at the DnaA boxes in either the promoter or terminator of the *TtDnaA* gene in *T. tengcongensis* [20], then accelerating RNAP reading through or termination.

Of course, the exact role of the interaction between TtDnaA and TtNusG2, in both transcription and replication, remains to be investigated. It would be helpful to address this novel phenomena and mechanism *in vivo* when a genetic manipulation system becomes available for *T. tengcongensis*.

Acknowledgments

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References

[1] W. Messer, The bacterial replication initiator DnaA. DnaA and oriC, the bacterial mode to initiate DNA replication, FEMS Microbiol. Rev. 26 (2002) 355–374.

[2] W. Messer, F. Blaesing, D. Jakimowicz, M. Krause, J. Majka, J. Nardmann, S. Schaper, H. Seitz, C. Speck, C. Weigel, G. Wegrzyn, M. Welzeck, J. Zakrzewska-Czerwinska, Bacterial replication initiator DnaA. Rules for DnaA binding and roles of DnaA in origin unwinding and helicase loading, Biochimie 83 (2001) 5–12.

[3] J.P. Erzberger, M.M. Pirruccello, J.M. Berger, The structure of bacterial DnaA: implications for general mechanisms underlying DNA replication initiation, EMBO J. 21 (2002) 4763–4773.
[4] A. Lobner-Olesen, K. Skarstad, F.G. Hansen, K. von Meyenburg, E. Boye, The DnaA protein determines the initiation mass of *Escherichia coli* K-12, Cell 57 (1989) 881–889.
[5] W. Messer, C. Weigel, DnaA as a transcription regulator, Methods Enzymol. 370 (2003) 338–349.
[6] W. Messer, C. Weigel, DnaA initiator-also a transcription factor, Mol. Microbiol. 24 (1997) 1–6.
[7] J.R. Knowlton, M. Bubunenko, M. Andrykovitch, W. Guo, K.M. Routzahn, D.S. Waugh, D.L. Court, X. Ji, A spring-loaded state of NusG in its functional cycle is suggested by X-ray crystallography and supported by site-directed mutants, Biochemistry 42 (2003) 2275–2281.
[8] R.J. Horwitz, J. Li, J. Greenblatt, An elongation control particle containing the N gene transcriptional antitermination protein of bacteriophage lambda, Cell 51 (1987) 631–641.
[9] J. Li, S.W. Mason, J. Greenblatt, Elongation factor NusG interacts with termination factor rho to regulate termination and antitermination of transcription, Genes Dev. 7 (1993) 161–172.
[10] S.W. Mason, J. Greenblatt, Assembly of transcription elongation complexes containing the N protein of phage lambda and the *Escherichia coli* elongation factors NusA, NusB, NusG, and S10, Genes Dev. 5 (1991) 1504–1512.
[11] S.L. Sullivan, M.E. Gottesman, Requirement for *E. coli* NusG protein in factor-dependent transcription termination, Cell 68 (1992) 989–994.
[12] S.L. Sullivan, D.F. Ward, M.E. Gottesman, Effect of *Escherichia coli* nusG function on lambda N-mediated transcription antitermination, J. Bacteriol. 174 (1992) 1339–1344.
[13] C.M. Burns, J.P. Richardson, NusG is required to overcome a kinetic limitation to Rho function at an intragenic terminator, Proc. Natl. Acad. Sci. USA 92 (1995) 4738–4742.
[14] E. Burova, S.C. Hung, V. Sagitov, B.L. Stitt, M.E. Gottesman, *Escherichia coli* NusG protein stimulates transcription elongation rates *in vivo* and *in vitro*, J. Bacteriol. 177 (1995) 1388–1392.
[15] C.L. Squires, J. Greenblatt, J. Li, C. Condon, C.L. Squires, Ribosomal RNA antitermination *in vitro*: requirement for Nus factors and one or more unidentified cellular components, Proc. Natl. Acad. Sci. USA 90 (1993) 970–974.
[16] M. Torres, J.M. Balada, M. Zellars, C. Squires, C.L. Squires, *In vivo* effect of NusB and NusG on rRNA transcription antitermination, J. Bacteriol. 186 (2004) 1304–1310.
[17] N.C. Kyrpides, C.R. Woese, C.A. Ouzounis, KOW: a novel motif linking a bacterial transcription factor with ribosomal proteins, Trends Biochem. Sci. 21 (1996) 425–426.
[18] Y. Xue, Y. Xu, Y. Liu, Y. Ma, P. Zhou, *Thermoanaerobacter tengcongensis sp. nov*., a novel anaerobic, saccharolytic, thermophilic bacterium isolated from a hot spring in Tengcong, China, Int. J. Syst. Evol. Microbiol. 51 (2001) 1335–1341.
[19] Q. Bao, Y. Tian, W. Li, Z. Xu, Z. Xuan, S. Hu, W. Dong, J. Yang, Y. Chen, Y. Xue, Y. Xu, X. Lai, L. Huang, X. Dong, Y. Ma, L. Ling, H. Tan, R. Chen, J. Wang, J. Yu, H. Yang, A complete sequence of the *T. tengcongensis* genome, Genome Res. 12 (2002) 689–700.
[20] H. Pei, J. Liu, J. Li, A. Guo, J. Zhou, H. Xiang, Mechanism for the TtDnaA-Tt-oriC cooperative interaction at high temperature and duplex opening at an unusual AT-rich region in *Thermoanaerobacter tengcongensis*, Nucleic Acids Res. 35 (2007) 3087–3099.
[21] S. Wang, J. Bajorath, D.B. Flies, H. Dong, T. Honjo, L. Chen, Molecular modeling and functional mapping of B7-H1 and B7-DC uncouple costimulatory function from PD-1 interaction, J. Exp. Med. 197 (2003) 1083–1091.
[22] W. Messer, F. Blaesing, J. Majka, J. Nardmann, S. Schaper, A. Schmidt, H. Seitz, C. Speck, D. Tungler, G. Wegrzyn, C. Weigel, M. Welzeck, J. Zakrzewska-Czerwinska, Functional domains of DnaA proteins, Biochimie 81 (1999) 819–825.
[23] M.D. Sutton, J.M. Kaguni, The *Escherichia coli dnaA* gene: four functional domains, J. Mol. Biol. 274 (1997) 546–561.
[24] T. Steiner, J.T. Kaiser, S. Marinkovic, R. Huber, M.C. Wahl, Crystal structures of transcription factor NusG in light of its nucleic acid- and protein-binding activities, EMBO J. 21 (2002) 4641–4653.
[25] C.J. Ingham, J. Dennis, P.A. Furneaux, Autogenous regulation of transcription termination factor Rho and the requirement for Nus factors in *Bacillus subtilis*, Mol. Microbiol. 31 (1999) 651–663.
[26] Z. Pasman, P.H. von Hippel, Regulation of rho-dependent transcription termination by NusG is specific to the *Escherichia coli* elongation complex, Biochemistry 39 (2000) 5573–5585.
[27] J. Li, R. Horwitz, S. McCracken, J. Greenblatt, NusG, a new *Escherichia coli* elongation factor involved in transcriptional antitermination by the N protein of phage lambda, J. Biol. Chem. 267 (1992) 6012–6019.
[28] T. Linn, J. Greenblatt, The NusA and NusG proteins of *Escherichia coli* increase the *in vitro* readthrough frequency of a transcriptional attenuator preceding the gene for the beta subunit of RNA polymerase, J. Biol. Chem. 267 (1992) 1449–1454.
[29] C.M. Burns, L.V. Richardson, J.P. Richardson, Combinatorial effects of NusA and NusG on transcription elongation and Rho-dependent termination in *Escherichia coli*, J. Mol. Biol. 278 (1998) 307–316.
[30] M.M. Bagdasarian, M. Izakowska, M. Bagdasarian, Suppression of the DnaA phenotype by mutations in the rpoB cistron of ribonucleic acid polymerase in *Salmonella typhimurium* and *Escherichia coli*, J. Bacteriol. 130 (1977) 577–582.
[31] T. Atlung, E.S. Clausen, F.G. Hansen, Autoregulation of the dnaA gene of *Escherichia coli* K12, Mol. Gen. Genet. 200 (1985) 442–450.
[32] R.E. Braun, K. O'Day, A. Wright, Autoregulation of the DNA replication gene dnaA in *E. coli* K-12, Cell 40 (1985) 159–169.
[33] C. Kucherer, H. Lother, R. Kolling, M.A. Schauzu, W. Messer, Regulation of transcription of the chromosomal dnaA gene of *Escherichia coli*, Mol. Gen. Genet. 205 (1986) 115–121.
[34] Q.P. Wang, J.M. Kaguni, Transcriptional repression of the *dnaA* gene of *Escherichia coli* by dnaA protein, Mol. Gen. Genet. 209 (1987) 518–525.
[35] F.G. Hansen, B.B. Christensen, T. Atlung, The initiator titration model: computer simulation of chromosome and minichromosome control, Res. Microbiol. 142 (1991) 161–167.