Universidade do Minho

INSTITUTE FOR BIOTECHNOLOGY AND BIOENGINEERING

INSTITUTE FOR BIOTECHNOLOGY AND BIOENGINEERING

BRINGING TEXT MINERS AND BIOLOGISTS CLOSER TOGETHER

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Outline

- Who are we?
 - Research and Development areas
 - Domains of expertise
- @ Note, our literature mining workbench:
 - Aims
 - Contributions
- Final remarks.

Nature Precedings doi:10.1038/npre.2009.3188.1 Posted 27 Apr 2009
Guanosine tetraphosphate (ppGpp) is a key mediator of stringent control, an adaptive response of bacteria to amino acid starvation, and has thus been termed a bacterial alarmone. Previous X-ray crystallographic analysis has provided a structural basis for the transcriptional regulation of RNA polymerase activity by ppGpp in the thermophilic bacterium *Thermus thermophilus*. Here we investigated the physiological basis of the stringent response by comparing the changes in intracellular levels and the rate of RNA synthesis in stringent (rel(+); wild type) and relaxed (rel(-); mutant) strains of *T. thermophilus*. We found that in wild-type *T. thermophilus*, as in other bacteria, serine hydroxamate, an amino acid analogue that inhibits tRNA aminoacylation, elicited a stringent response characterized in part by intracellular accumulation of ppGpp and that this response was completely blocked in a relA-null mutant and partially blocked in a relC mutant harboring a mutation in the ribosomal protein L11. Subsequent in vitro assays using ribosomes purified from wild-type and relA and relC mutant strains confirmed that (p)ppGpp is a potent inhibitor of RNA polymerase and that mutation of RelA or L11 blocks that activity. This conclusion was further supported in vitro by demonstrating that thioesteron or tetracycline inhibit (p)ppGpp synthesis. In contrast, the system (p)ppGpp induced by heat shock catalyzed 23S / 5S rRNA gene transcription but at a concentration much higher than that of the observed intracellular ppGpp. On the other hand, changes in the rRNA gene promoter activity were correlated with changes in the GTP but not ATP concentration. Also, (p)ppGpp exerted a potent inhibitory effect on IMP dehydrogenase activity. The present data thus complement the earlier structural analysis by providing physiological evidence that *T. thermophilus* does produce ppGpp in response to amino acid starvation in a ribosome-dependent (i.e., RelA-dependent) manner. However, it appears that in *T. thermophilus*, rRNA promoter activity is controlled directly by the GTP pool size, which is modulated by ppGpp via inhibition of IMP dehydrogenase activity. Thus, unlike the case of *Escherichia coli*, ppGpp may not inhibit *T. thermophilus* RNA polymerase activity directly in vivo, as recently proposed for *Bacillus subtilis* rRNA transcription (L. Krasny and R. L. Gourse, EMBO J. 23:4473 - 4483, 2004).

http://sysbio.uminho.pt

ShortBiology

Escherichia coli
Zymomonas mobilis
Kluyveromyces fragilis
Streptococcus pneumoniae

Optimization of fed-

Guanosine tetraphosphate (ppGpp) is a key mediator of stringent control, an adaptive response of bacteria to amino acid starvation, and has thus been termed a bacterial alarmone. Previous X-ray crystallographic analysis has provided a structural basis for the transcriptional regulation of RNA polymerase activity by ppGpp in the thermophilic bacterium *Thermus thermophilus*. Here we investigated the physiological basis of the stringent response by comparing the changes in intracellular ppGpp levels and the rate of RNA synthesis in stringent (rel(+); wild type) and relaxed (rel(-) and relC; mutant) strains of *T. thermophilus*. We found that in wild-type *T. thermophilus*, as in other bacteria, serine hydroxamate, an amino acid analogue that inhibits tRNA (Ser) aminoacylation, elicited a stringent response characterized in part by intracellular accumulation of ppGpp and that this response was completely blocked in a relA-null mutant and partially blocked in a relC mutant harboring a mutation in the ribosomal protein L11. Subsequent in vitro assays using ribosomes purified from wild-type and relA and relC mutant strains confirmed that (p)ppGpp is a potent inhibitor of RNA polymerase and that mutation of RelA or L11 blocks that activity. This conclusion was further supported in vitro by demonstrating that thioesteron or tetracycline inhibit (p)ppGpp synthesis. In contrast, the system (p)ppGpp induced by heat shock catalyzed 23S / 5S rRNA gene transcription but at a concentration much higher than that of the observed intracellular ppGpp. On the other hand, changes in the rRNA gene promoter activity were correlated with changes in the GTP but not ATP concentration. Also, (p)ppGpp exerted a potent inhibitory effect on IMP dehydrogenase activity. The present data thus complement the earlier structural analysis by providing physiological evidence that *T. thermophilus* does produce ppGpp in response to amino acid starvation in a ribosome-dependent (i.e., RelA-dependent) manner. However, it appears that in *T. thermophilus*, rRNA promoter activity is controlled directly by the GTP pool size, which is modulated by ppGpp via inhibition of IMP dehydrogenase activity. Thus, unlike the case of *Escherichia coli*, ppGpp may not inhibit *T. thermophilus* RNA polymerase activity directly in vivo, as recently proposed for *Bacillus subtilis* rRNA transcription (L. Krasny and R. L. Gourse, EMBO J. 23:4473 - 4483, 2004).

@NOTE

Biological Literature
Mining (BIM)
System s
Biology
informatics
<http://sysbio.dh.minho.pt/anote/wiki>

Heterogeneous data integration

Open-source plugins

Customised end-user applications

PubMed Search

Keeping with routine... but making
some things easier...

Evaluating paper relevance,
organising papers,...

Managing Lexical Resources

User is in charge of the annotation process!!!

Selects the sources of terminology, determines the entity classes to be annotated,...

Dictionaries can be re-used and combined

Genomic rephosphorylation (ppp-ppp) is a key regulator of stringent control, an adaptive response of bacteria to amino acid starvation, and has thus been termed a bacterial hormone. Previous 2-ep crystallographic analysis has provided a structural basis for the transcriptional regulation of *ppp-ppp* polyphosphate activity by *ppp-ppp* in the *thermophilus* bacterium *Thermus thermophilus*. Here we investigated the physiological basis of the stringent response by comparing the changes in intracellular levels and the rate of *ppp-ppp* synthesis in stringent (rel⁺, wild-type) and relaxed (rel⁻, and *ppp-ppp* deficient) strains of *T. thermophilus*. We found that in wild-type *T. thermophilus*, as in other *rel⁺* strains, *ppp-ppp* synthesis is induced by amino acid starvation, and that *ppp-ppp* synthesis is induced by amino acid starvation in a *rel⁺* mutant and partially blocked in *ppp-ppp* deficient strains.

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AI Bench
File Database Get Publication
Clipboard
Clipboard
ANoteProject
anoteProject
Lexical Resou
Dictionary
Lookup Ta
ANoteProjectConnec
ANoteProjectCon

Manual Curation

User takes the final decision!!!

Ensures annotation quality,
refines lexical resources,...

Entity Frequency per Biological Classes

User does not deal with annotation back-end...

User-friendly multi-purpose curation + Construction of gold standard corpora

Next Step: Extracting Relations...

Again, back-end and front-end address different concerns...

User-friendly multi-purpose
curation + Construction of gold
standard corpora

heat	the originally examined response	response	response
heat shock	associated 12,50%	0,00 %	100,00 %
	to be 12,50%	0,00 %	100,00 %

Gammaproteobacteria (*ppg-pp*) is a key regulator of stringent control, an adaptive response of bacteria to amino acid starvation, and has thus been termed a bacterial hormone. Previous 2-ep crystallographic analysis has provided a structural basis for the transcriptional regulation of *PDC* polymerase activity by *ppg-pp* in the thermophilic bacterium *Thermus thermophilus*. Here we investigated the physiological basis of the stringent response by comparing the changes in intracellular levels and the rate of *PDC* synthesis in stringent (rel⁻), wild-type (rel⁺) and related (rel⁺) and *ppg-pp* mutant strains of *T. thermophilus*. We found that in wild-type *T. thermophilus*, as in other *γ*, *ppg-pp* hydrolyses, an amino acid analogue that inhibits *PDC* accumulation of aminoacyl-tRNA^{Pro} elicited a stringent response characterized in part by intracellular accumulation of *ppg-pp* and this response was completely blocked in a *relA*-null mutant and partially blocked in *relB* mutants. Providing a mutation in the ribosomal protein L11. Subsequent in vitro assays using ribosomes isolated from wild-type and *relA* and *relB* mutant strains confirmed that (p) *ppg-pp* is *synthetase* ribosomes and that mutation of *RelA* or *L11* blocks that activity. This conclusion was further supported in vitro by demonstrating that this response or *relA* inhibits (p) *ppg-pp* *synthetase* in a *ppg-pp* mutant in vitro system. (p) *ppg-pp* acted by inhibiting *PDC* polymerase-activated SS/SS *PDC* transcription but at a concentration much higher than that of the observed intracellular *ppg-pp* pool size. On the other hand, changes in the *PDC* gene promoter activity tightly correlated changes in the *GTP* but not *ATP* concentration. Also, (p) *ppg-pp* exerted a potent inhibitory effect on *GTP* dehydrogenase activity. The present data thus complemented the earlier structural analysis by providing physiological evidence that *T. thermophilus* does produce *ppg-pp* in response to amino acid starvation in a ribosome-dependent (i.e., *RelA*-dependent) manner. However, it appears that in *T. thermophilus*, *PDC* promoter activity is controlled directly by the *GTP* pool size, which is modulated by *ppg-pp* via inhibition of *GTP* dehydrogenase activity. Thus, unlike the case of *Escherichia coli*, *ppg-pp* may not inhibit *T. thermophilus* *PDC* polymerase activity directly in vivo, as recently proposed for *Bacillus subtilis* *PDC* transcription (J. Koenig and R. L. Gourse, EMBO J. 24:4475–4483, 2004).

FINAL REMARKS

Cooperation with other Literature Mining groups

Real-world applications

Guanine tetraphosphate (*ppGpp*) is a key indicator of stringent control, an adaptive response of bacteria to amino acid starvation, and has thus been termed a bacterial alarmone. Previous 7-mer crystallographic analyses has provided a structural basis for the transcriptional regulation of RNA polymerase activity by *ppGpp* in the thermophilic bacterium *Thermus thermophilus*. Here we investigated the physiological basis of the stringent response by comparing the changes in intracellular levels and the rate of *tRNA_{Ser}* synthesis in stringent (wt) wild-type) and relaxed (*relA*) and *relB* mutant strains of *T. thermophilus*. We found that in multiple *T. thermophilus*, as in other Gram-positive bacteria, an amino acid analogue that inhibits RelA/GSce aminoacyltransferase elicited a stringent response characterized in part by intracellular accumulation of *ppGpp* and whose response was completely blocked in a *relA*-null mutant and partially blocked in a *relB* mutant carrying a mutation in the ribosomal protein L11. Subsequent *in vitro* assays using ribosomes purified from three wild-type and *relA* and *relB* mutant strains confirmed that (i) *ppGpp* is synthetized by RelA/GSceases and that mutation of *RelA* or L11 blocks that activity; this conclusion was further supported *in vitro* by demonstrating that thioesterase inhibitors (ii) *ppGpp* synthetizes a *ppGpp* pool size; (iii) *ppGpp* acted by inhibiting *RelA*'s polymerase-aided SS rRNA transcription but at a concentration much higher than that of the observed intracellular *ppGpp* pool size. On the other hand, changes in the *relA* gene promoter activity slightly overrode changes in the *GTP* but not *ATP* concentrations. Also, (iv) *ppGpp* exerted a potent inhibitory effect on *DAP* dehydrogenase activity. The present data thus complement the earlier conclusions by providing physiological evidence that *T. thermophilus* does produce *ppGpp* in response to amino acid starvation in a ribosome-dependent (i.e., *RelA*-dependent) manner. However, it appears that in *T. thermophilus*, *relA* promoter activity is controlled directly by the *GTP* pool size, which is modulated by *ppGpp* via inhibition of *DAP* dehydrogenase activity. Thus, unlike the case of *Escherichia coli*, *ppGpp* may not inhibit *T. thermophilus* *RelA* polymerase activity directly *in vivo*, as recently proposed for *Bacillus subtilis* *RelA* transcription [L. Koss and R. Gerner, EMBO J. 24(47):4493–4498, 2004].

BioPSEg
BioProcess Systems Engineering
group

[HTTP://SYSBIO.DI.UMINHO.PT/ANOTE/WIKI](http://sysbio.di.uminho.pt/anote/wiki)

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