JOURNAL OF BACTERIOLOGY, Oct. 2000, p. 5911–5915 Vol. 182, No. 20
0021-9193/00/$04.00+0

Organization and Expression of a *Thermus thermophilus* Arginine Cluster: Presence of Unidentified Open Reading Frames and Absence of a Shine-Dalgarno Sequence

RONY SANCHEZ,[1] MARTINE ROOVERS,[1] AND NICOLAS GLANSDORFF[1,2,3]*

Department of Microbiology, Flanders Interuniversity Institute for Biotechnology (VIB),[1] Department of Microbiology, Free University of Brussels (VUB),[2] and Research Institute J. M. Wiame,[3] 1070 Brussels, Belgium

Received 18 April 2000/Accepted 26 July 2000

A group of genes regulated by arginine was found clustered in the order *argF*-ORF1-*argC*-*argJ*-ORF4 between other, as yet uncharacterized, open reading frames (ORFs). Transcription starts were identified immediately upstream from *argF* and ORF4. Arginine repressed transcription that was initiated at *argF* but induced transcription of ORF4. The functions of ORF1 and ORF4 are unknown, but analysis of the sequence of ORF4 suggests that it is a membrane protein, possibly involved in transport of arginine or a related metabolite. Mobility shift and DNase I footprinting have revealed specific binding of pure *Escherichia coli* ArgR to the promoter region of *Thermus thermophilus argF*. These results suggest that *argF* transcription is controlled by a repressor homologous to those characterized in enteric bacteria and bacilli. *Thermus argF* mRNA is devoid of Shine-Dalgarno (SD) sequences. However, downstream from the ATG start codon of *argF* and many other *Thermus* genes (with or without an SD box), sequences were found to be complementary to nucleotides 1392 to 1409 of *Thermus* 16S rRNA, suggesting that an mRNA-rRNA base pairing in this region is important for correct translation initiation.

In arginine biosynthesis two alternative pathways have evolved to split off the acetyl group of *N*-acetylornithine. *Enterobacteriaceae*, *Vibrionaceae*, *Myxococcus xanthus*, and possibly also the archaeon *Sulfolobus acidocaldarius* use a linear pathway in which the formation of ornithine is mediated by acetyl ornithinase (encoded by *argE*) (14, 12, 31, 36). Other bacteria, archaea, and eukaryotic microbes recycle the acetyl group by transacetylation of *N*-acetylornithine and glutamate (26). The transacetylation is catalyzed by ornithine acetyltransferase (encoded by *argJ*), an enzyme which in some organisms is also able to use acetyl coenzyme A to acetylate glutamate and in this way bypasses the first step of the linear pathway (11, 26). Ornithine carbamoyltransferase (encoded by *argF*) converts ornithine and carbamoyl phosphate (CP) into citrulline. CP is extremely thermolabile, and in *Pyrococcus furiosus* (17), *Pyrococcus abyssi* (25), and *Thermus thermophilus* ZO5 (32), it appears to be protected from thermal decomposition into the indiscriminate carbamoylating agent cyanate, by channeling towards citrulline and carbamoyl aspartate.

Arginine genes may be either scattered (as in several proteobacteria, *Aquifex aeolicus*, cyanobacteria, and archaea) or clustered in two different patterns (2, 36): divergently transcribed clusters (enteric bacteria and *Vibrionaceae*) or clusters of variable extension where *argC* and *argJ* are found together, as in the gram-positive organisms *Thermotoga maratima* and *Thermus* (see below). Nevertheless, regulation appears to be similar in most of these organisms: an ArgR repressor interacts with specific operator sequences (called Arg boxes) overlapping the promoter region (5, 6, 12, 18, 28). Homologous proteins were also reported to activate genes involved in arginine degradation in bacilli (19, 20). However, a nonhomologous arginine regulatory protein has been identified in *Pseudomonas aeruginosa* (22). Except for proteobacteria and gram-positive organisms, little is known about the regulation of arginine biosynthesis. *T. thermophilus* (24) is an interesting bacterium in two respects, as a paradigm for investigations on extreme thermophily and as a representative of a deep-branching division which also contains *Deinococcus radiodurans* (its closest relative), *Chloroflexus*, and *Thermomicrobium* (34). In this study we describe the organization and expression of an unusual type of arginine gene clustering in *T. thermophilus* HB27.

For all experiments, *Thermus* was grown at 75°C in arginine- and uracil-free liquid medium (4) with 20 mM pyruvate as the carbon source and 10 mM ammonium sulfate as the nitrogen source. Chromosomal DNA partially digested with *Sau3A* was used to construct a λ-ZAP genomic library (Stratagene), which was screened as described by Sanchez et al. (27). Primer extension experiments were performed according to the method described by Kholti et al. (15) except that hybridization experiments were performed overnight at 45°C. Primers were oligonucleotides complementary to the sequences 60 to 80 nucleotides downstream from the ATG codon of each open reading frame (ORF). S1 nuclease mapping was also done according to the method described by Kholti et al. (15). For mobility shift assays, pure *Escherichia coli* and *Bacillus stearothermophilus* arginine repressors (final concentration, 45 μg/ml) were incubated for 30 min at 37°C with a ^{32}P-end-labeled DNA fragment in binding buffer (1 mM Tris-HCl [pH 7.4], 5 mM $MgCl_2$, 250 mM KCl, 2.5 mM $CaCl_2$, 0.5 mM dithiothreitol, and 2.5% glycerol) with a 100-fold excess of sonicated herring sperm DNA in the presence or absence of 10 mM arginine. DNA-protein complexes were then immediately loaded on a 6% polyacrylamide gel. For DNase I footprinting experiments (10) a single ^{32}P-end-labeled DNA fragment (100 ng/ml) and a 100-fold excess of nonspecific competitor DNA were incubated for 30 min at 37°C in binding buffer (as for the mobility shift assay described above) containing pure repressor (9 μg/ml). DNase I was added at a final concentration of 0.7 mg/ml, and

* Corresponding author. Mailing address: Flanders Interuniversity Institute for Biotechnology (VIB), Free University of Brussels (VUB), and Research Institute J. M. Wiame, E. Grysonlaan 1, 1070 Brussels, Belgium. Phone: 32 2 5267275. Fax: 32 2 5267273. E-mail: ceriair@ulb.ac.be.

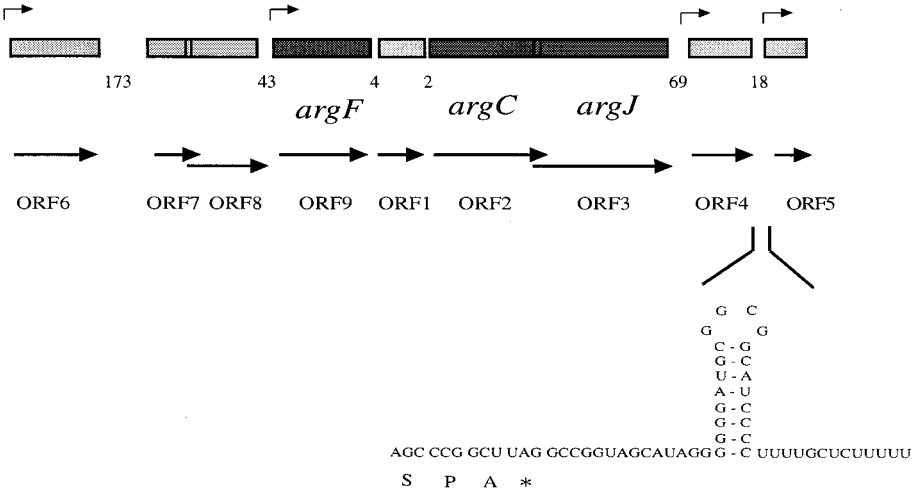


FIG. 1. Schematic drawing of the organization of arginine biosynthetic genes in *T. thermophilus*. The genes involved in arginine biosynthesis are: *argF* (ornithine carbamoyltransferase), *argC* (*N*-acetyl-gamma-glutamyl-phosphate reductase), and *argJ* (glutamate *N*-acetyltransferase). Other ORFs encode proteins with unknown functions. Arrows show the direction and starting points of transcription. The number of nucleotides present between each pair of ORFs is indicated. A putative rho-independent terminator is present between ORF4 and ORF5. The deduced C-terminal amino acid sequence of ORF4 is shown; the stop codon is indicated by an asterisk.

the digestion was terminated after 30 s by the addition of stop buffer (0.6 M ammonium acetate and 0.05 M EDTA [final concentration]) and 10 μg of yeast tRNA. After DNA precipitation the reaction products were analyzed on a 6% denaturating polyacrylamide gel.

Clustering of arginine biosynthetic genes and unidentified ORFs. By screening the λ-ZAP library we identified in order of transcription ORF6-ORF7-ORF8-*argF* (i.e., ORF9)-ORF1-*argC* (i.e., ORF2)-*argJ* (i.e., ORF3)-ORF4-ORF5 (Fig. 1). The start codon of *argJ* overlaps the stop codon of *argC*. This is also the case for ORF7 and ORF8. A putative rho-independent terminator was found between ORF4 and ORF5. A BLAST search showed that ORF6-like proteins belong to the UPF0078 family (National Center for Biotechnology Information database). They occur in several bacteria, such as *E. coli* (YgiH), *Bacillus subtilis* (YneS), and *Mycoplasma pneumoniae* (YgiH), as potential integral membrane proteins containing several putative transmembrane regions. At least five such regions occur in ORF6 (data not shown). Alignment studies showed highly conserved regions in the N-terminal (GATN) and central parts (FKGGKAVAT) of the protein. ORF7 encodes a polypeptide of 113 amino acids; it is homologous to five genes scattered throughout the genome of *Methanococcus jannaschii*, one of which was found next to *carB*, which encodes a subunit of CP synthetase. Two exemplars of this gene were found in *Synechocystis*. These ORF7 homologues all overlap a neighboring gene. In *Thermus*, ORF7 overlaps ORF8. ORF8-like genes occur in *E. coli* (*yaiS*), *B. subtilis* (*ypjG*), *Mycobacterium leprae* (*lmbE*), and *Streptomyces lincolnensis* (*lmbE*). Few genes homologous to ORF1 or ORF4 were found. An ORF1 homologue occurs in *Synechocystis* with 32% identity at the amino acid level. For ORF4, a similar gene was encountered in *D. radiodurans* (31% identical amino acids). Interestingly, the N-terminal part of ORF4 is similar to the signal sequence of outer membrane proteins of the OmpA family (Fig. 2). No similarity was found beyond the signal sequence. ORF4 thus probably encodes a membrane protein; in this respect it is reminiscent of the *P. aeruginosa* OpcD porin (23), which facilitates the diffusion of basic amino acids. There is no obvious similarity between the two sequences, but expression of both OpcD and *Thermus* ORF4 (see below) is strongly induced by arginine.

Starting points of transcription. Primer extension experiments performed for each of the genes present in the cluster showed that transcription was initiated just before ORF4 (Fig. 2), ORF5, and ORF6 (data not shown), whereas no transcription starts were observed immediately upstream of ORF7,

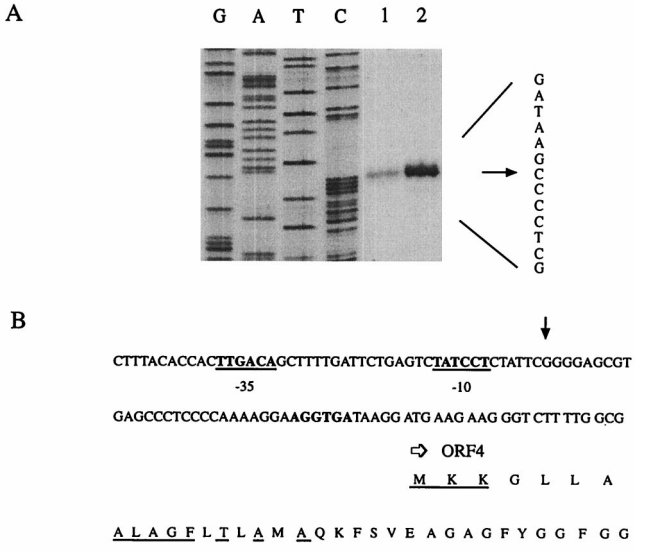


FIG. 2. (A) Primer extension mapping of the transcriptional starting point of the *T. thermophilus* ORF4 region. Equal amounts (about 50,000 cpm) of 5′-end-labeled 20-mer primer were mixed with 100 μg of RNA extracted from cultures grown in minimal medium (1) or supplemented with arginine (2). After precipitation and hybridization the extension reaction was performed. The position of the transcript is indicated by the arrow; the sequence shown is of the noncoding strand. Lanes G, A, T, and C represent chain-terminating DNA-sequencing reactions of the noncoding strand with the oligonucleotide also used as a primer in the primer extension experiment. (B) Nucleotide and deduced amino acid sequences of a portion of the *T. thermophilus* ORF4 gene and its promoter region. The arrow pointing downward indicates the transcriptional start site. Also, −35 and −10 promoter sequences are underlined and in bold type. A putative ribosome binding site is indicated by bold letters. The underlined amino acids of ORF4 are identical to the signal sequence of seven proteins of the OmpA family (*E coli*, *Salmonella enterica* serovar Typhimurium, *B. subtilis*, *Pseudomonas luteolum*, *Serratia marcescens*, *Enterobacter aerogenes*, and *Shigella dysenteriae*).

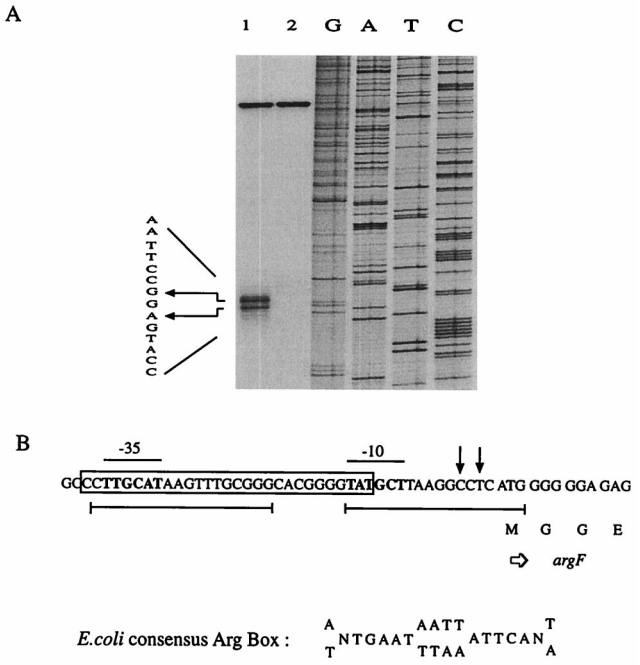


FIG. 3. (A) S1 nuclease mapping of the transcriptional starting point of the *argF* region. Equal amounts (50,000 cpm) of the 5′-end-labeled 150-bp PCR fragment containing the promoter region of *argF* were hybridized with RNA extracted from cultures grown in minimal medium (lane 1) and minimal medium supplemented with arginine (lane 2). After precipitation and hybridization, S1 nuclease activity proceeded. The position of the transcript is indicated by the arrows; the sequence shown is of the noncoding strand. Lanes G, A, T, and C represent chain-terminating DNA-sequencing reactions of the noncoding strand. (B) Nucleotide and deduced amino acid sequences of a portion of the *T. thermophilus argF* gene and its promoter region. The arrows indicate the transcriptional start sites. Also, −35 and −10 promoter sequences are indicated and in bold type. Sequences showing similarity to the *E. coli* consensus Arg box are underlined. Boxed sequences were protected from DNase I treatment.

ORF8, ORF1, *argC*, or *argJ*. Transcription from the ORF6 promoter was very weak and not influenced by arginine, whereas transcription of ORF4 was strongly activated in the presence of arginine. In primer extension experiments, *argF* cDNA synthesis stopped within the coding region, presumably because of a secondary structure forming in the transcript. S1 nuclease mapping showed two major bands (Fig. 3) corresponding to a T residue and a C residue, 2 and 4 nucleotides upstream from the AUG codon, respectively. The intensity was strongly reduced with RNA extracted from cells grown in the presence of arginine. Previous experiments already indicated that arginine represses OTCase synthesis in *Thermus* (27, 31). The region thus appears to contain four consecutive transcription units: ORF6, ORF7, ORF8, *argF*; ORF1, *argC*, *argJ*; ORF4; and ORF5.

In vitro binding of the *E. coli* arginine repressor to the *Thermus argF* promoter region. *Thermus argF* promoter sequences overlapping the −35 and −10 elements exhibit similarity to the palindromic sequence of the *E. coli* consensus Arg box; the sequence overlapping the −35 region shows up to 62% identity (Fig. 4). We studied the interaction of pure *E. coli* ArgR and *B. stearothermophilus* AhrC with the promoter region of *Thermus argF* and ORF4 by mobility shift electrophoresis and footprinting. In the presence of arginine, *E. coli* ArgR binds to the promoter region of *Thermus argF* (Fig. 4), where it protects a 29-nucleotide region from DNase I digestion (Fig. 5). This region contains the first putative Arg box covering the −35 region and part of the second Arg box (Fig. 4). The latter is not completely protected by *E. coli* ArgR, probably because of the

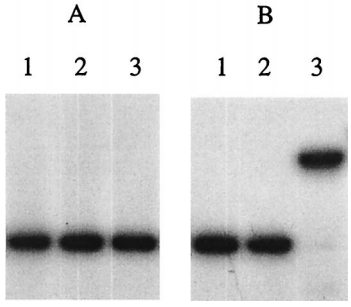


FIG. 4. Mobility shift experiments for the *Thermus argF* promoter region with *E. coli* ArgR and *B. stearothermophilus* AhrC in the absence (A) and presence (B) of arginine. Lanes l, no added protein; lanes 2, *argF* promoter fragment incubated with *B. stearothermophilus* AhrC; lanes 3, *argF* promoter fragment incubated with *E. coli* ArgR.

7-bp spacing of the two boxes (3). The *B. stearothermophilus* Arg repressor did not interact with the *argF* promoter. No arginine repressor was reported so far in *Thermus*, but in the genome of *D. radiodurans* there is an ORF encoding a protein clearly homologous to the Arg repressor characterized in enteric bacteria and bacilli. Thus, it appears that *Thermus argF* is controlled by such a repressor. No specific interaction between the *E. coli* and *B. stearothermophilus* arginine repressors could be demonstrated with the *Thermus* ORF4 promoter region (data not shown). Further studies should show whether repression of *argF* and induction of ORF4 involve the same *Thermus* protein.

Translation initiation signals. The *argF* mRNA is devoid of a Shine-Dalgarno (SD) sequence. Some mRNAs lacking SD sequences were found in bacteria, archaea, eukarya (35), and eukaryotic organelles (21), suggesting that information within the coding sequence may be sufficient to signal the translational start in diverse biological systems. In several genes of *E. coli* and of *E. coli* bacteriophages, Sprengart et al. (29) identified sequences complementary to nucleotides 1469 to 1483 of 16S rRNA. This region is exposed on the ribosome surface and expected to come into contact with the 3′ end of 16S RNA. Mutations were found in the *E. coli gln* gene which increase the complementarity of downstream sequences to 16S rRNA and result in higher translational activity (8). We found that 64% of 130 analyzed genes from *Thermus* (including *argF*) showed such a downstream box, where at least 6 consecutive nucleo-

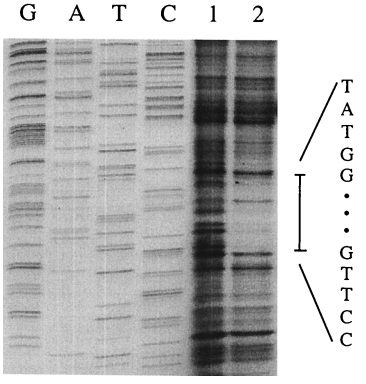
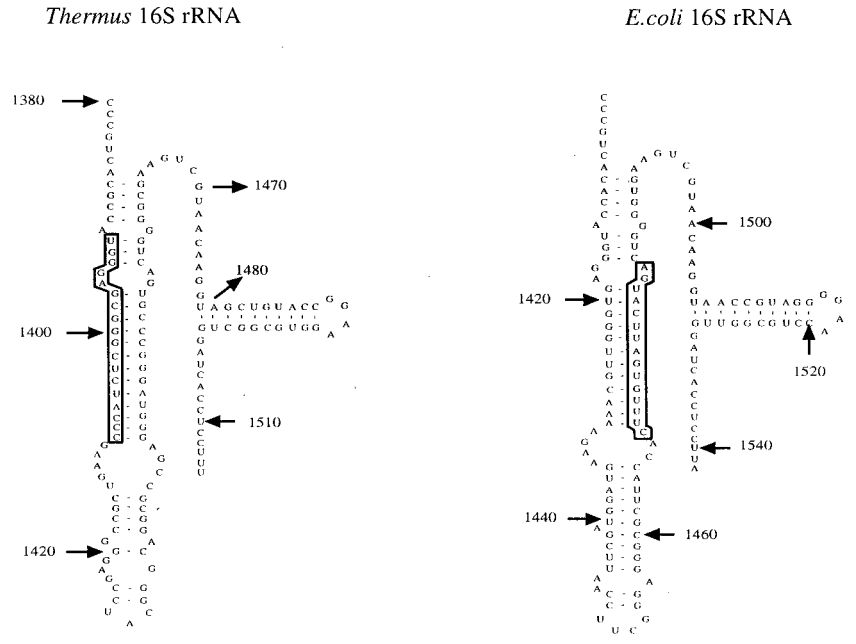


FIG. 5. DNase I footprinting of the 210-bp fragment of the *Thermus argF* promoter region protected by the *E. coli* arginine repressor. Lanes: G, A, T, and C, sequencing ladders; 1, DNase I reference ladder (without *E. coli* ArgR); 2, DNase I treatment in the presence of *E. coli* ArgR. The approximately 29-bp-long protected stretch is indicated by a bar.

A



B

16S RNA Consensus sequence (1418) C C G C U G A A **G C C C A U C U C G G G C G A G G G U** A C
(anti downstream box)

Genes with a Shine-Dalgarno sequence.

polA	G G A G U A G C A U G G A G G C G A U G C U U C C G C U C U U U G A
orB	G G A G U C U G A G A U G C U G G A G C U C A A G C U U G C G
orA	A A A G G G G G G A A A A U G G A G G A G U U C A C C U G G C C C
pol	G G A G U A G C A U G G A G G C G A U G C U U C C G C U
argJ	A A G G A G G G G U U A U G G C C G U G A G G C U U C C C A G
xylA	A G G A G G U G A U G U A G G G U G U A C G A G C C C A A A C C G G A G
sodA	G G A G G U G A A G U A U G C C G U A C C C G U U C A A G C U U C C U G A

Genes without a Shine-Dalgarno sequence.

lysS	A U G A A C G A C C A G A C C C G C C A G C G C C U G C U C A A C C U
Unknown (Y15464)	A U G C U C A C C U G G G U G G A C C U C C U G G C C C U G
ppa	A U G G C G A A C C U G A A G A G C C U U C C C G U G G G
pyrH	A U G A A G U A C A A G C G G G U U C U C C U G
cp	G U G A C G C C G G A A G C C G C U U A U C A G
ldh	G U G A A G G U C G G C A U C G U G G G U A G C G G C U U C G U G G G G A
pro	G U G A G G C C A C C C C A U C G G C U U G C G C C C G C A U G G G G G
argF	A U G G G G G G A G A G G C C C U G A C C C U G

FIG. 6. (A) Sequences at the 3′ ends of *Thermus* and *E. coli* 16S rRNAs. The region of the 16S RNA molecule complementary to sequences downstream of the AUG start codon of several *Thermus* and *E. coli* genes is indicated by boxed nucleotides. (B) Examples of "downstream boxes" in genes from *Thermus*. Sequences complementary to the region of 16S rRNA shown above are in bold type and underlined. The SD sequence is underlined, and the start codon is indicated by a grey box throughout.

tides or at least 8 nucleotides out of 12 complemented the nucleotide-1392-to-1409 region of *Thermus* 16S rRNA (Fig. 6). This sequence differs from the one mentioned by Sprengart et al. (29, 30) but is situated in a similarly exposed stem-loop structure (Fig. 6). This consensus sequence was found in *Thermus* mRNAs containing an SD sequence as well as in mRNAs lacking one. These observations suggest that an mRNA-rRNA interaction at the level of these downstream sequences could in some cases compensate for absent SD boxes or reinforce interactions with existing ones.

Concluding remarks. Clusters of functionally related genes containing apparently unrelated ORFs have been reported in other organisms (7, 11). It was claimed that the inclusion of genes in unrelated operons may be selectively neutral if the latter are constitutive (16). This is clearly not the case for ORF1, which is coregulated with *argF*, *argC*, and *argJ*. One may argue that the product of coregulated ORFs could stabilize enzymes encoded by the same polycystronic mRNA (13). However, in *Thermus*, the *argF* and *argJ* gene products appear intrinsically stable (1, 27). Another possibility would be that the unknown ORF product is involved in the formation of multienzyme complexes channeling arginine metabolic precursors (17, 32). The situation in *D. radiodurans*, the closest known relative of *Thermus*, is also intriguing. *Deinococcus argF* is followed at 8 bp by a homologue of *mutT* (encoding an enzyme degrading 8-oxo-dGTP). This *mutT* gene overlaps *argC*, itself followed at 4 nucleotides by the gene for glycerol-3-phosphate dehydrogenase (33). In *Deinococcus*, however, the regulation of those genes has not yet been studied. We feel that the inclusion of unknown or already identified "foreign" genes into defined operons is a neglected area of molecular physiology in need of further investigation.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper are available in the EMBL Nucleotide Database under accession number Y18353.

This work was supported by a grant from the Flanders Scientific Foundation for Scientific Research (FWO).

We thank D. Charlier for the gift of pure arginine repressor and are grateful for the excellent help of Nadine Huysveld.

REFERENCES

1. **Baetens, M., C. Legrain, A. Boyen, and N. Glansdorff.** 1998. Genes and enzymes of the acetyl cycle of arginine biosynthesis in the extreme thermophilic bacterium *Thermus thermophilus* HB27. Microbiology **144:**479–492.
2. **Bringel, F., L. Frey, S. Boivin, and J. C. Hubert.** 1997. Arginine biosynthesis and regulation in *Lactobacillus plantarum*: the *carA* gene and the *argCJBDF* cluster are divergently transcribed. J. Bacteriol. **179:**2697–2706.
3. **Charlier, D., M. Roovers, F. Van Vliet, A. Boyen, R. Cunin, Y. Nakamura, N. Glansdorff, and A. Pierard.** 1992. Arginine regulon of *Escherichia coli* K12. A study of repressor-operator interactions and of in vitro binding affinities versus in vivo repression. J. Mol. Biol. **226:**367–386.
4. **Degryse, E., N. Glansdorff, and A. Piérard.** 1978. A comparative analysis of extreme thermophilic bacteria belonging to the genus *Thermus*. Arch. Microbiol. **177:**189–196.
5. **Dimova, D., P. Weigel, M. Takahashi, F. Marc, G. D. Van Duyne, and V. Sakanyan.** 2000. Thermostability, oligomerisation and DNA-binding properties of the regulatory protein ArgR from the hyperthermophilic bacterium *Thermotoga neapolitana*. Mol. Gen. Genet. **263:**119–130.
6. **Dion, M., D. Charlier, H. Wang, D. Gigot, A. Savchenko, J. N. Hallert, N. Glansdorff, and V. Sakanyan.** 1997. The highly thermostable arginine repressor of *Bacillus stearothermophilus*: gene cloning and repressor-operator interactions. Mol. Microbiol. **25:**385–398.
7. **Fani, R., P. Lio, and A. Lazeano.** 1995. Molecular evolution of the histidine biosynthetic pathway. J. Mol. Evol. **41:**760–774.
8. **Faxén, M., J. Plumbridge, and L. A. Isaksson.** 1991. Codon choice and potential complementarity between mRNA downstream of the initiation codon and bases 1471–1480 in 16S ribosomal RNA affects expression of *glnS*. Nucleic Acid Res. **19:**5247–5251.
9. **Fried, M., and D. Crothers.** 1983. CAP and RNA polymerase interaction with the *lac* promoter: binding stoichiometry and long range effects. Nucleic Acids Res. **11:**141–158.
10. **Galas, D. J., and A. Schmitz.** 1978. DNase footprinting: a simple method for the detection of protein-DNA binding specificity. Nucleic Acids Res. **5:**3157–3170.
11. **Gifford, C. M., and S. S. Wallace.** 2000. The genes encoding endonuclease VIII and endonuclease III in *Escherichia coli* are transcribed as the terminal genes in operons. Nucleic Acids Res. **28:**762–769.
12. **Glansdorff, N.** 1996. Biosynthesis of arginine and polyamines, p. 408–433. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 1. American Society for Microbiology, Washington, D.C.
13. **Glansdorff, N.** 1999. On the origin of operons and their possible role in evolution towards thermophily. J. Mol. Evol. **49:**432–438.
14. **Harris, B. Z., and M. Singer.** 1998. Identification and characterization of the *Myxococcus xanthus argE* gene. J. Bacteriol. **180:**6412–6414.
15. **Kholti, A., D. Charlier, D. Gigot, N. Huysveld, M. Roovers, and N. Glansdorff.** 1998. *pyrH*-encoded UMP-kinase directly participates in pyrimidine-specific modulation of promoter activity in *Escherichia coli.* J. Mol. Biol. **280:**571–582.
16. **Lawrence, J. G., and J. R. Roth.** 1996. Selfish operons: horizontal transfer may drive the evolution of gene clusters. Genetics **143:**1843–1860.
17. **Legrain, C., M. Demarez, N. Glansdorff, and A. Piérard.** 1995. Ammonia-dependent synthesis and metabolic channeling of carbamoyl phosphate in the hyperthermophilic archaeon *Pyrococcus furiosus*. Microbiology **141:**1093–1099.
18. **Maas, W. K.** 1994. The arginine repressor of *Escherichia coli.* Microbiol. Rev. **58:**631–640.
19. **Maghnouj, A., T. Franco de Sousa Cabral, V. Stalon, and C. Vander Wauven.** 1998. The *arcABDC* gene cluster, encoding the arginine deiminase pathway of *Bacillus licheniformis*, and its activation by the arginine repressor ArgR. J. Bacteriol. **180:**6468–6475.
20. **Miller, C. M., S. Baumberg, and P. G. Stockley.** 1997. Operator interactions by the *Bacillus subtilis* arginine repressor/activator, AhrC: novel positioning and DNA-mediated assembly of a transcriptional activator at catabolic sites. Mol. Microbiol. **26:**37–48.
21. **Montoya, J., D. Ojala, and G. Attardi.** 1981. Distinctive features of the 5′-terminal sequences of the human mitochondrial mRNAs. Nature **290:**465–470.
22. **Nishijyo, T., S.-M. Park, C.-D. Lu, Y. Itoh, and A. T. Abdelal.** 1998. Molecular characterization and regulation of an operon encoding a system for transport of arginine and ornithine and the ArgR regulatory protein in *Pseudomonas aeruginosa.* J. Bacteriol. **180:**5559–5566.
23. **Ochs, M. M., C. D. Lu, R. E. Hancock, and A. T. Abdelal.** 1999. Amino acid-mediated induction of the basic amino acid-specific outer membrane porin OprD from *Pseudomonas aeruginosa*. J. Bacteriol. **181:**5426–5432.
24. **Oshima, T., and K. Imahori.** 1974. Description of *Thermus thermophilus* (Yoshima and Oshima) comb. nov., a nonsporulating thermophilic bacterium from a Japanese thermal spa. Int. J. Syst. Bacteriol. **24:**102–112.
25. **Purcarea, C., D. R. Evans, and G. Herve.** 1999. Channeling of carbamoyl phosphate to the pyrimidine and arginine biosynthetic pathways in the deep sea hyperthermophilic archaeon *Pyrococcus abyssi*. J. Biol. Chem. **274:**6122–6129.
26. **Sakanyan, V., P. Petrosyan, M. Lecocq, A. Boyen, C. Legrain, M. Demarez, J. N. Hallet, and N. Glansdorff.** 1996. Genes and enzymes of the acetyl cycle of arginine biosynthesis in *Corynebacterium glutamicum*: enzyme evolution in the early steps of the arginine pathway. Microbiology **142:**99–108.
27. **Sanchez, R. A., M. Baetens, M. van de Casteele, M. Roovers, C. Legrain, and N. Glansdorff.** 1997. Ornithine carbamoyltransferase from the extreme thermophile *Thermus thermophilus*. Analysis of the gene and characterisation of the protein. Eur. J. Biochem. **248:**466–477.
28. **Soutar, A., and S. Baumberg.** 1996. Implication of a repression system, homologous to those of other bacteria, in the control of arginine biosynthesis genes in *Streptomyces coelicolor*. Mol. Gen. Genet. **251:**245–251.
29. **Sprengart, M. L., H. P. Fatscher, and E. Fuchs.** 1990. The initiation of translation in *E. coli*: apparent base pairing between the 16S RNA and downstream sequences of the mRNA. Nucleic Acids Res. **18:**1719–1723.
30. **Sprengart, M. L., and A. G. Porter.** 1997. Functional importance of RNA interactions in selection of translation initiation codons. Mol. Microbiol. **24:**19–28.
31. **Van de Casteele, M., M. Demarez, C. Legrain, N. Glansdorff, and A. Piérard.** 1990. Pathways of arginine biosynthesis in extreme thermophilic archaea and eubacteria. J. Gen. Microbiol. **136:**1177–1183.
32. **Van de Casteele, M., C. Legrain, L. Demarez, P. G. Chen, A. Piérard, and N. Glansdorff.** 1997. Molecular physiology of carbamoylation under extreme conditions: what can we learn from extreme thermophilic microorganisms? Comp. Biochem. Physiol. **188A:**463–473.
33. **White, O., J. A. Eisen, J. F. Heidelberg, E. K. Hickey, J. D. Peterson, et al.** 1999. Genome sequence of the radioresistant bacterium *Deinococcus radiodurans* R1. Science **286:**1571–1577.
34. **Woese, C. R.** 1987. Bacterial evolution. Microbiol. Rev. **51:**221–271.
35. **Wu, C. J., and G. R. Janssen.** 1996. Translation of vph mRNA in *Streptomyces lividans* and *Escherichia coli* after removal of the 5′ untranslated leader. Mol. Microbiol. **22:**339–355.
36. **Xu, Y., Z. Liang, C. Legrain, H. J. Rüger, and N. Glansdorff.** 2000. Evolution of arginine biosynthesis in the bacterial domain: novel gene-enzyme relationships from psychrophilic *Moritella* strains (*Vibrionaceae*) and the evolutionary significance of *N*-α-acetyl ornithinase. J. Bacteriol. **182:**1609–1615.