Journal of Bioinformatics and Computational Biology
Vol. 3, No. 4 (2005) 1–13

Imperial College Press
www.icpress.co.uk

EVOLUTION OF THE NADR REGULON IN ENTEROBACTERIACEAE

ANNA V. GERASIMOVA*

Laboratory of Bioinformatics, State Scientific Center GOSNIIGenetika
1-iy Dorozhny proezd 1, Moscow, 113545, Russia
a_gerasimova@yahoo.com

MIKHAIL S. GELFAND

Institute for Information Transmission Problems, RAS
Bolshoy Karetny per. 19, Moscow, GSP-4, 127994, Russia
gelfand@iitp.ru

Received 5 February 2005
Revised 18 February 2005
Accepted 24 February 2005

The NAD biosynthetic pathway and NAD transformations in *E. coli* and *S. typhi* are well characterized. Using comparative genomics methods we describe the NadR regulon in other *Enterobacteriaceae*, identity new candidate regulon members and demonstrate that even a very simple regulon covering an essential methabolic pathway could be different in closely related genomes.

Keywords: NAD biosynthesis; NadR; transcription factor; regulation of transcription; comparative genomics; phylogenetic footprinting; evolution.

1. Introduction

The comparative approach to the analysis of regulation is based on the assumption that regulons are conserved in related bacteria containing ortologous transcription factors.

This approach, reviewed in Refs. 1–3, has been successfully applied to the analysis of many regulatory systems[4–15] and served as a base for large-scale analyses of regulation in all prokaryotes,[16,17] as well as selected taxonomic groups of gamma-proteobacteria,[18,19] delta-proteobacteria,[20] and gram-positive bacteria,[21,22] resulting in identification of numerous new signals and functional annotation of tens of hypothetical genes. Many of such predictions were subsequently confirmed in experiment,[23,24,12] or even served as a starting point for experimental

*Corresponding author.

analysis.[18,25–27] There exist several Internet servers for comparative analysis of bacterial regulation, in particular, EnteriX[28] and PredictRegulon.[29]

In an attempt to analyze the evolutionary dynamics of a relatively simple, well-studied regulon that includes genes from an essential part of the metabolism, we considered the NadR regulon in *Enterobacteriaceae*.

The nicotinamide adenine dinucleotides (NAD, NADH, NADP, NADPH) are essential cofactors in all living systems and function as hydride acceptors (NAD, NADP) and donors (NADH, NADPH) in biochemical redox reactions.[30] At high internal levels of NAD, the transcriptional regulator NadR represses the *de novo* synthesis and salvage pathways. NadR is a multifunctional protein, consisting of an N-terminal DNA-binding domain which represses NAD biosynthesis, a central nicotinamide mononucleotide adehyltransferase (NMNAT) domain and a C-terminal RNK domain.[31,32]

The NAD biosynthetic pathway and transformations are shown in Fig. 1.[31]

Genes known to be repressed by NadR in *E. coli* and *S. typhi* are marked by rectangles. These are two NAD biosynthesis genes, *nadA* and *nadB*, and a niacin salvage gene *pncB*.[32,33]

2. Data and Methods

The complete genomes of *Escherichia coli* K-12 MG1655[34] (EC), *Shigella flexneri* 2457T[35] (SF), *Salmonella typhi* CT18[36] (ST), *Erwinia carotovora* subsp. *atroseptica* SCRI1043[37] (ERW), *Yersinia pestis* CO92[38] (YP) and *Photorhabdus luminescens* subsp. *laumondii* TT01[39] (PHL) were obtained from Genbank.[40]

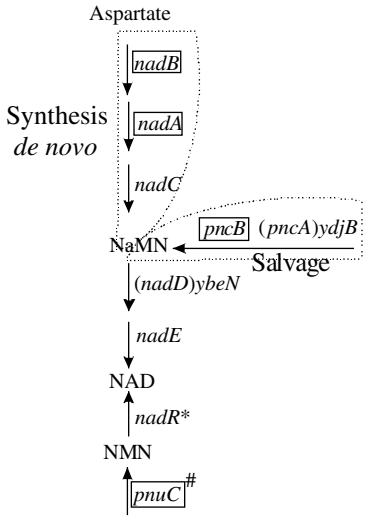

Fig. 1. The NAD biosynthetic pathway and transformations in *Enterobacteriaceae*.
Notation: "*": enzymatic domain; "#": NMN transporter, regulated within the *nadApnuC* operon.

Incomplete genomes of *Klebsiella pneumoniae* MGH78578 (KP) and *Serratia marcescens* Db11 (SM) were downloaded from the websites of the Washington University Consortium (www.genome.wustl.edu), and *Yersinia enterocolitica* 8081 (YE), from the Sanger Institute website (www.sanger.ac.uk).

Profiles (positional weight matrices) for the identification of candidate NadR-binding sites were constructed using SignalX.[4] The training set consists of upstream regions of *nadA* from *E. coli, S. typhi* and *Y. pestis, nadB* from *E. coli* and *S. typhi*, and *pncB* from *E.coli, S.typhi* and *Y. pestis.*

Sequence logo was constructed using WebLogo.[41] Orthologs were identified by the bidirectional best hits criterion[42] and, if necessary, verified by construction of phylogenetic trees using PHYLIP.[43] Multiple nucleotide and protein alignments were constructed using ClustalX.[44] Genome analyses were performed using GenomeExplore.[45]

3. Results and Discussion

NadR orthologs were identified in all studied Enterobacteria. Multiple protein alignment demonstrated that NadR orthologs in all considered genomes contained DNA-binding domain, NMNAT domain and RNK domain.

It is known that in some gamma-proteobacteria, for example in *Haemophilus influenzae*, NadR orthologs do not contain the DNA-binding domain[31] and thus have only enzymatic, but not regulatory role. Indeed, no DNA-binding domains were found in NadR orthologs from genomes outside the *Enterobacteriaceae* and *Pasteurellaceae* families. Among the latter, *Haemophilus influenzae* is the only genome with NadR lacking the DNA-binding domain. NadR of other *Pasteurellaceae* have the DNA-binding domain, but these genomes have no *nadA*, *nadB* and *pncB* orthologs, nor do they have candidate sites for the enterobacterial NadR-signal. Thus here we restricted the analysis to the NadR regulon in *Enterobacteriaceae.*

The recognition profile was constructed as described above. The sequence logo of the NadR signal is shown in Fig. 2.

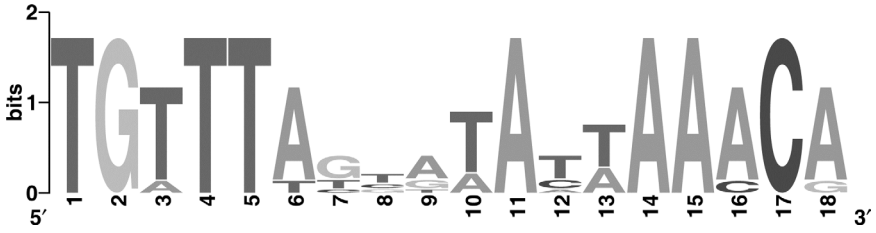

Fig. 2. Sequence logo of NadR-sites from the training set. The total height of the symbols in each position equals the positional information content, whereas the height of individual symbols is proportional to the positional nucleotide frequency, with the most frequent nucleotide shown at the top.

Table 1. Genes from candidate NadR regulons.

	nadB		nadA		pncB		nadR		ynfL/M		rpsP	
Genome	Name of Ortologues Gene	Score	Name of Ortologues Gene	Score	Name of Ortologues Gene	Score	Name of Ortologues Gene	Score	Name of Ortologues Gene	Score	Name of Ortologues Gene	Score
EC	nadB	6.21	nadA	5.95	pncB	5.63	nadR	—	ynfL/M	4.69	rpsP	—
SF	in DNA	6.21	nadA	5.95	pncB	5.63	nadR	—	ynfL/M	4.69	rpsP	—
ST	STY2834	6.21 5.09	STY0797	5.95	STY1010	6.06	nadR	— —	STY1578/79	—	STY2863	—
KP	nadB	6.21	nadA	5.95	pncB	5.11	nadR	—	ynfL/M	4.69	in DNA	—
ERW	nadB	—	ECA1378	4.62 5.23	pncB	—	ECA0463	5.62	ECA2259/60	4.69	ECA3359	5.10
SM	nadB	—	nadA	5.17 5.81	pncB	5.07	nadR	5.71	ynfL/M	4.69	in DNA	5.16
YP	nadB	—	nadA	4.29 5.86	pncB	4.62	nadR	5.91	in DNA/YPO2266	5.33	rpsP	5.16
YE	RYE01420	—	RYE03344	5.86	RYE02025	—	RYE00967	5.63	RYE00573/74	4.69	RYE01243	5.16
PHL	nadB	—	plu1468	6.43	pcnB	—	nadR	—	plu2225/24	5.33	rpsP	4.80

Notation: "+": gene with a candidate NadR-site in the upstream region; "–": gene without NadR-sites; "0": no ortholog.
*The number of candidate sites in the genome in the interval (–300) bp to (+10) bp relative to the gene start. Sites scoring higher than 4.6 are considered. No overlap with the upstream gene is allowed.

The signal is a palindrome with six conserved positions at each side and a spacer of six relatively less conserved positions.

The study started with identification of orthologs of genes that constitute the NadR regulon in *E. coli* and analysis of their regulation. The results are shown in Table 1.

NadR-sites of the *nadA* genes are conserved and they form the only conserved island in the alignment of upstream regulons (Fig. 3).

Additional candidate sites were identified in *S. marcescens* *E. carotovora.*

```
EC|   TGTAAACCTGATGACATCGTCAGAGCTTACTGTGCAAGCAACTCTA-TGTC-GGTGGAAT
SF|   TGTAAACCTGATGACATCGTCAGAGCTTACTGTGCAAGCAACTCTA-CGTC-GGTGGAAT
KP|   CG--GTCACGCTGCGCTTACCGT-GCCTGCACAGGATACGAACCGGTCGTTCGGGCAGAT
ST|   ----GATGCGCTGCGCTTATCAG-GCCTACAGACGGCACCGCTCA--CGTA-GGCCAGAT
Plu|  ----AATAAATAGCTTTAATA----AA-ACAGCTTTTATAAATA-----TAATAATGTTT
YP|   ----GGTTTCTTTCCTCACTC----TCTACTGATACGTGAATTA-----TCACCTCGTTT
YE|   ----AACAGATCTCTATAATCCTCTACTATCCTTTCTATCACTCAA--CTTCCCCCATAT
ERW|  CTTGTCTCTCTCCAACTGATAATACG-----ACCCACGCGTTTCA----CCTTATTATTTT
SM|   TACGTTCTCCTCAGGCTGTTAATCCGCTGTGAACTGCGCGGCTAA----TGCCTATGTTT
                                                          *

EC|   TAGGCGTAAAATGACGCATCC---TGCACATTAGGCGTAATTCGA-GTGACTTTTCCCCA
SF|   TAGGCGTAA------------------------------TTCGA-GTGACTTTTCCCCA
KP|   GCGCAGCACCGTCCCCGGGAAAAGGGTCTGATGTACAAAATTCATCACGACTTTTAGCTC
ST|   AAGACGTTACGCCGCCATCA----GATACGGACTACGTAATT-ATTGTGACTTTCCTTAT
Plu|  T-----CTGTTTAGCCATCCT----ATCATAGAT--AAAATATCTCGCGATAAATTACTA
YP|   TTAACTCTATTAAACATTTTC----GCTATTAATCGACAAATTTTGGCGACTTTTTGCCA
YE|   TTAACTCTATTAAACATTTTT----GCCATTTTTAGACAATATCTGGCGACTTTTCGCAA
ERW|  TAAGCATAGTAAACAAAGTGG---TGGGTTTTTTGGAAAAAACACCGCGACTTTAGGGCG
SM|   TTAATCTATTAAACATTGAGT---GCGCAAAATTGAAAATATCAGCGGACTTTTCGCGCG

                    TGTTTA      TAAACA
EC|   CCATTCGACTATCTTGTTTAGCATATAAAACAAATTACACCGA-TAACAGCGAATA---T
SF|   CCATTCGACTATCTTGTTTAGCATATAAAACAAATTACACCGA-TAACAGCGAATG---T
KP|   GTAATCGGCTATCTTGTTTAGCATATAAAACACCATGACCGTA-TTAAGCCGCCGA---C
ST|   TGAATCAGCTATCTTGTTTAGCATATAAAACAAATTGACCG-A-TTGTGGCGTTTA---T
Plu|  TAAATTGATCATTTTGTTTAGTATATTAAACAAATCTGGGCAAATTTT-CGGACTG---A
YP|   AATATTGGTTATTTTGTTTAGGATAAAAAACAAATCAATTCAATTCTTACTGGGTGGCTA
YE|   AATATTGAGTATTTTGTTTAGGATAAAAAACAAATCAATCTGA--CTGGCAGCCTG---A
ERW|  TGTTTTCGGTATTTTGTTTAGGATAAAAAACTCC-------GATGCTGATAG--------
SM|   -ATTTTAGGTATTTTGTTTAGTATATAAAACGAA-------GGGCGTTGCGG--------
          *     ** ******** ***  ****                  *

EC|   TACGC-TAATGTCGGTTTTA--ACGTTAAGCCTGTAAAACGAGA--TGGTAAGATGAGCG
SF|   TACGC-TAATGTCGGTTTTA--ACGTTAAGCCTGTAAAACGAGA--TGGTAAGATGAGCG
KP|   AGGCCATACTGACGGTTATA--GAGTTAAGCCAGTAAAACGAGA--TTGCATGATGAGCG
ST|   TACGCTTTCATTCGGTTGTT--TCGTTAAGTCAGTAAAACGAGA--AGCCACGATGAGCG
Plu|  AAACCAAAATTATCGCTTTT-TAAAACCATATACTAAACAGAGAGTCAAAGTAATGAACC
YP|   GCAGCGATCTGATAGCTTCAGTAATACCACAGC---AACAGAGA-TGTGAGCGATGAGCG
YE|   AAGGCA---TTATGGCTCCG---GAATCACTGGC--AATAGAGA-TGTGAGCGATGAGCG
ERW|  TAGACCAGTATCAGGTGTTA-CAATCCGAGTTGTCATCGTGGAA-ATCCA-TAATGAATA
SM|   TAGGCGATATTCACGCGGCA-GGCGCCCAGC--CTAGCATGAGA-TTGCAGCGATGAGTG
          *         *             *           *  *         ****
```

Fig. 3. Conservation of NadR-sites upstream of *nadA*. The sites are shadowed; positions conforming to the signal consensus and start codons (ATG) are set in boldface.

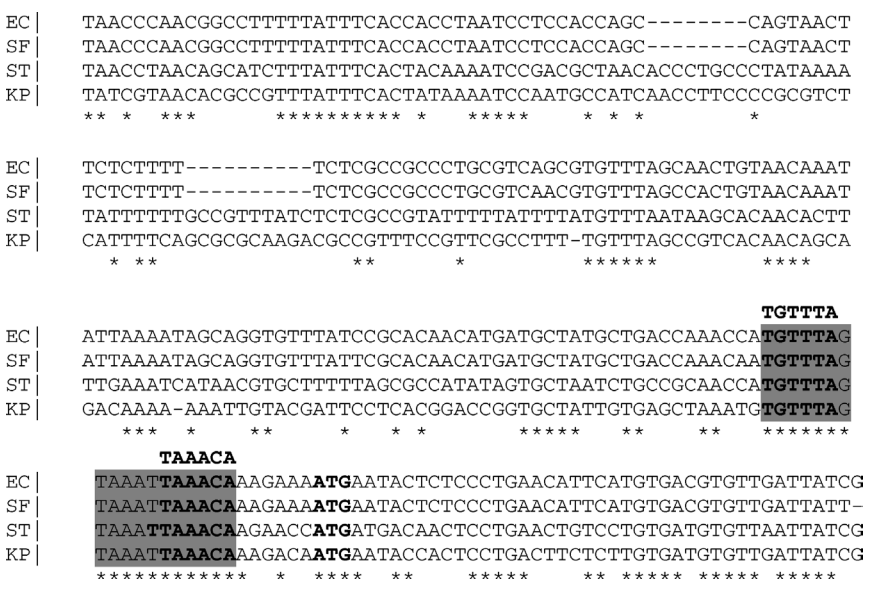

Fig. 4. Conservation of NadR-sites upstream of *nadB*. Notation as in Fig. 3.

Unexpectedly, NadR-sites upstream of other regulon members are not well conserved in genomes other than *S. typhi* and *E. coli*.

The NadR-site upstream of *nadB* is conserved in *E. coli, Sh. flexneri, S. typhi*, and *K. pneumoniae* (Fig. 4).

The corresponding regions of other genomes are not conserved, nor they contain candidate NadR-sites.

The situation with *pncB* is somewhat more interesting (Fig. 5a).

The site is conserved in *E. coli, Sh. flexneri* and *S. typhi*. The corresponding region in *K. pneumoniae* and *S. marcescens* is not conserved, although there are two conservation islands on both sides. Thus the NadR sites were destroyed in these genomes. New candidate sites appeared instead and these new sites do not seem to originate from local duplications. Indeed, there is no sequence conservation around "old" and "new" NadR-sites (Fig. 5b).

No sites were found in the remaining genomes.

In an attempt to find new candidate members of the NadR regulon, we identified candidate sites and considered all genes with candidate sites in at least four genomes. Unexpectedly, one of such genes was *nadR* itself, that had a strong candidate site in *E. carotovora, S. marcescens, Y. pestis* and *Y. enterocolitica*. The alignment of the upstream regions is shown in Fig. 6.

The "four-genome" condition holds in two more cases: two genes *ynfL* and *ynfM* transcribed in opposite directions, and *rpsP*.

The gene *ynfl* encodes a putative regulator from the LysR family, whereas *ynfM* encodes a putative transporter. We identified *ynfLM* ortjologs in *Pseudomonas* spp.

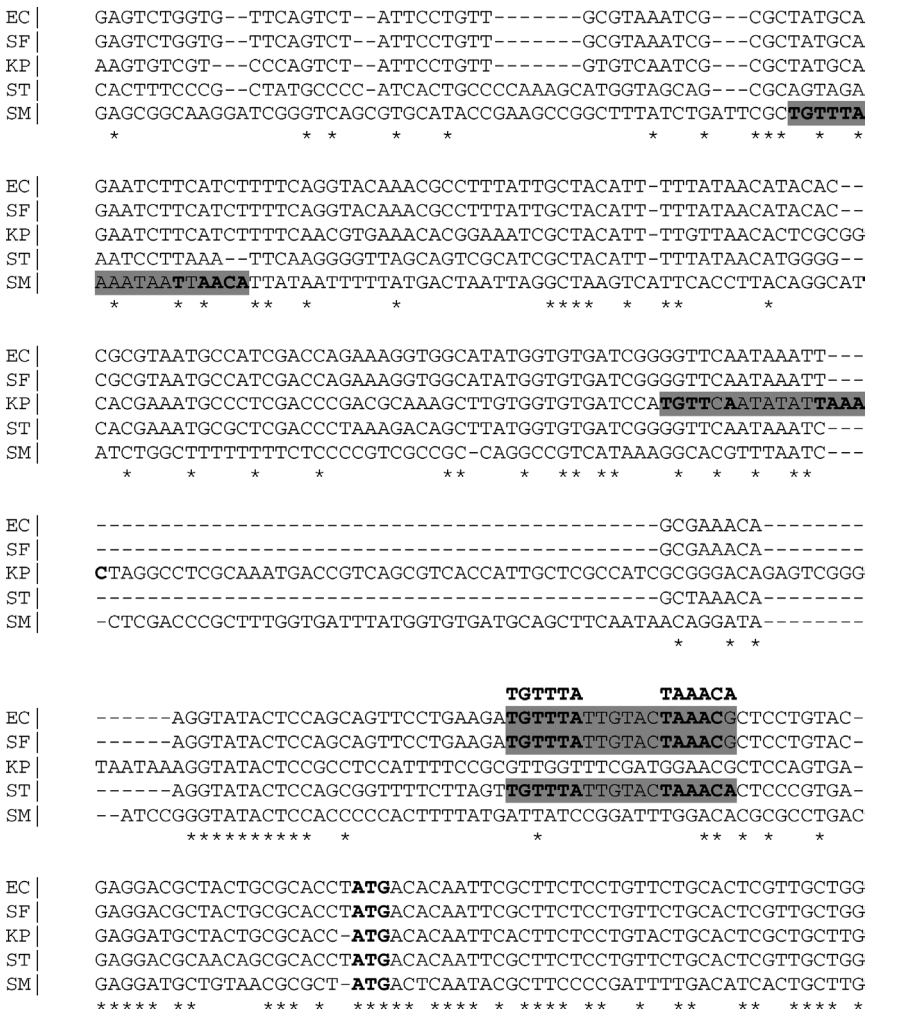

Fig. 5a. Conservation of "old" NadR-sites upstream of *pncB*. Notation as in Fig. 3.

Fig. 5b. Alignment of "new" NadR-sites upstream of *pncB*. Notation as in Fig. 3.

Fig. 6. Alignment of regions upstream of *nadR*. Notation as in Fig. 3.

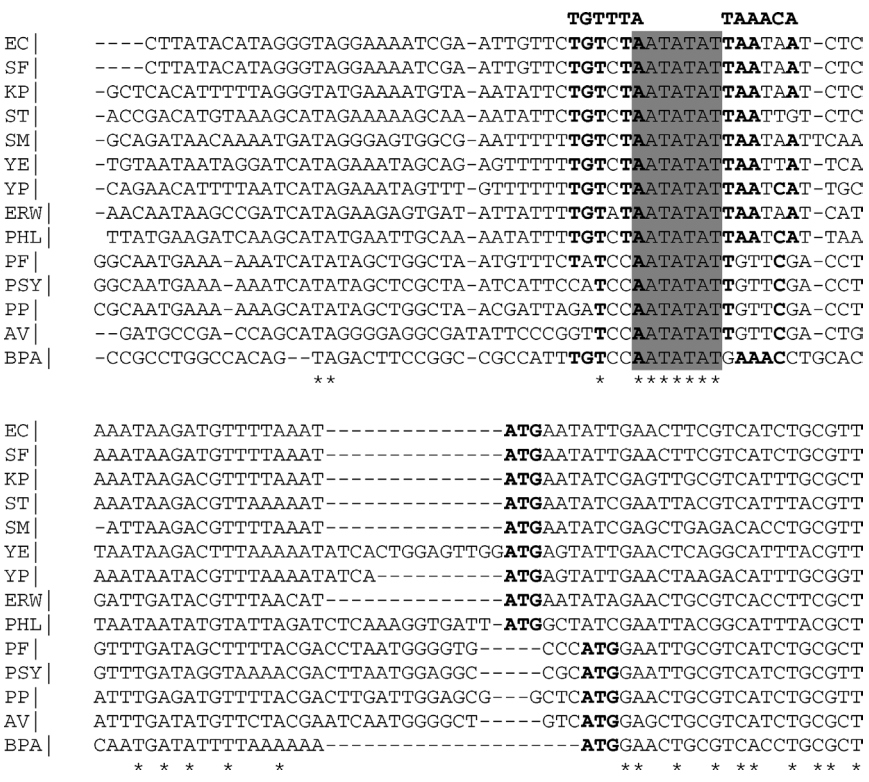

Fig. 7. Alignment of regions upstream of *ynfL*. Notation as in Fig. 3.
Notation: "PF" — *Pseudomonas fluorescens CHA0*, "PSY" — *Pseudomonas syringae*, "PP" — *Pseudomonas putida*, "AV" — *Azotobacter vinelandii*, "BPA" — *Bordetella parapertussis*.

and in *Bordetella parapertussis* and constructed multiple alignment of the intergenic region in all considered genomes (Fig. 7).

The conserved region coincides with the spacer of the candidate NadR binding site. On the other hand, there is no NadR regulator in *B. parapertussis* and in

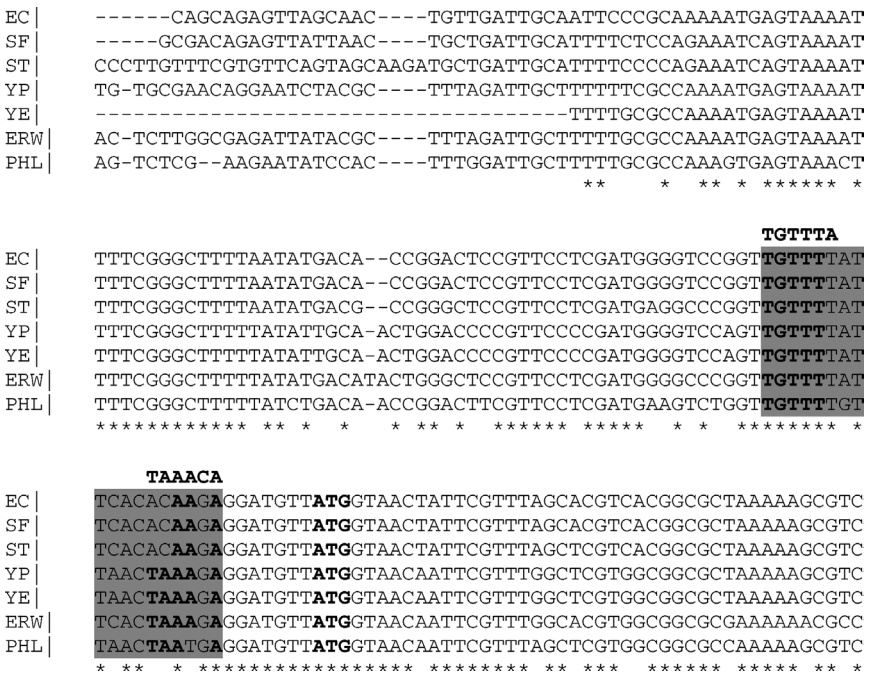

Fig. 8. Alignment of regions upstream of *rpsP*. Notation as in Fig. 3.

Pseudomonas spp., and thus this region cannot be a NadR-site. Since the arrangement where a binding site occurs between a divergently transcribed regulator gene and a regulated operon is very common, we conclude that the conserved region is the YnfL binding site. However, it is a very tentative prediction, requiring an experimental verification.

The gene *rpsP* encodes small ribosomal subunit protein S16. The nucleotide sequence of the *rpsP* upstream regions is uniformly conserved (Fig. 8).

This fact and the function of RpsP makes it unlikely that the observed site is functional.

4. Conclusions

This study demonstrated that even a very simple regulon covering an essential methabolic pathway could be different in closely related genomes. Not only the set of regulated genes can vary, but the autoregulation of the *nadR* gene by NadR, predicted here for the first time, is a feature of several, but not all genomes.

One of the possible explanations could be that the NadR regulon itself is rather young, as it exists in only one family of gamma-proteobacteria. However, the same behavior was observed for a number of other regulons, in particular Lrp,[46,47]

FruR,[46] KdgR.[25] More sequenced genomes are needed to elucidate the exact history of the NadR regulon.

Acknowledgments

We are grateful to Andrei Osterman, Dmitry Rodionov, Dmitry Ravcheev and Gavin H. Thomas for useful discussions.This study was partially supported by grants from the Howard Hughes Medical Institute (55000309) and Russian Academic of Sciences (Programs "Molecular and Cellular Biology" and "Origin and Evolution of the Biosphere").

References

1. Gelfand MS, Novichkov PS, Novichkova ES, Mironov AA, Comparative analysis of regulatory patterns in bacterial genomes, *Brief Bioinform* **1**:357–371, 2000.
2. Stojanovic N, Florea L, Riemer C, Gumucio D, Slightom J, Goodman M, Miller W, Hardison R, Comparison of five methods for finding conserved sequences in multiple alignments of gene regulatory regions, *Nucleic Acids Res* **27**:3899–3910, 1999.
3. Gelfand MS, Computational identification of regulatory sites in DNA sequences, in Frasconi P, Shamir R (eds.), *Artificial Intelligence and Heuristic Methods in Bioinformatics*, San-Miniato, Italy, IOS Press, pp. 148–172, 2003.
4. Mironov AA, Koonin EV, Roytberg MA, Gelfand MS, Computer analysis of transcription regulatory patterns in completely sequenced bacterial genomes, *Nucleic Acids Res* **27**: 2981–2989, 1999.
5. Gelfand MS, Koonin EV, Mironov AA, Prediction of transcription regulatory sites in Archaea by a comparative genomic approach, *Nucleic Acids Res* **28**:695–705, 2000.
6. Tan K, Moreno-Hagelsieb G, Collado-Vides J, Stormo GD, A comparative genomics approach to prediction of new members of regulons, *Genome Res* **11**:566–584, 2001.
7. Makarova KS, Mironov AA, Gelfand MS, Conservation of the binding site for the arginine repressor in all bacterial lineages, *Genome Biol* **2**:RESEARCH0013, 2001.
8. Laikova ON, Mironov AA, Gelfand MS, Computational analysis of the transcriptional regulation of pentose utilization systems in the gamma subdivision of Proteobacteria, *FEMS Microbiol Lett* **205**:315–322, 2001.
9. Panina EM, Mironov AA, Gelfand MS, Comparative analysis of FUR regulons in gamma-proteobacteria, *Nucleic Acids Res* **29**:5195–5206, 2001.
10. Permina EA, Mironov AA, Gelfand MS, Damage-repair error-prone polymerases of eubacteria: association with mobile genome elements, *Gene* **293**:133–140, 2002.
11. Rodionov DA, Mironov AA, Gelfand MS, Conservation of the biotin regulon and the BirA regulatory signal in Eubacteria and Archaea, *Genome Res* **12**:1507–1516, 2002.
12. Panina EM, Mironov AA, Gelfand MS, Comparative genomics of bacterial zinc regulons: enhanced ion transport, pathogenesis, and rearrangement of ribosomal proteins, *Proc Natl Acad Sci U S A* **100**:9912–9917, 2003.
13. Liu J, Tan K, Stormo GD, Computational identification of the Spo0A-phosphate regulon that is essential for the cellular differentiation and development in Gram-positive spore-forming bacteria, *Nucleic Acids Res* **31**:6891–6903, 2003.
14. Permina EA, Gelfand MS, Heat shock (sigma32 and HrcA/CIRCE) regulons in beta-, gamma- and epsilon-proteobacteria, *J Mol Microbiol Biotechnol* **6**:174–181, 2003.
15. Erill I, Escribano M, Campoy S, Barbe J, In silico analysis reveals substantial variability in the gene contents of the gamma proteobacteria LexA-regulon, *Bioinformatics* **19**:2225–2236, 2003.

16. Manson McGuire A, Church GM, Predicting regulons and their cis-regulatory motifs by comparative genomics, *Nucleic Acids Res* **28**:4523–4530, 2000.
17. McGuire AM, Hughes JD, Church GM, Conservation of DNA regulatory motifs and discovery of new motifs in microbial genomes, *Genome Res* **10**:744–757, 2000.
18. McCue L, Thompson W, Carmack C, Ryan MP, Liu JS, Derbyshire V, Lawrence CE, Phylogenetic footprinting of transcription factor binding sites in proteobacterial genomes, *Nucleic Acids Res* **29**:774–782, 2001.
19. Rajewsky N, Socci ND, Zapotocky M, Siggia ED, The evolution of DNA regulatory regions for proteo-gamma bacteria by interspecies comparisons, *Genome Res* **12**:298–308, 2002.
20. Rodionov DA, Dubchak I, Arkin A, Alm E, Gelfand MS, Reconstruction of regulatory and metabolic pathways in metal-reducing delta-proteobacteria, *Genome Biol* **5**:R90, 2004.
21. Terai G, Takagi T, Nakai K, Prediction of co-regulated genes in Bacillus subtilis on the basis of upstream elements conserved across three closely related species, *Genome Biol* **2**:RESEARCH0048, 2001.
22. Alkema WB, Lenhard B, Wasserman WW, Regulog analysis: detection of conserved regulatory networks across bacteria: application to Staphylococcus aureus, *Genome Res* **14**:1362–1373, 2004.
23. Rodionov DA, Mironov AA, Rakhmaninova AB, Gelfand MS, Transcriptional regulation of transport and utilization systems for hexuronides, hexuronates and hexonates in gamma purple bacteria, *Mol Microbiol* **38**:673–683, 2000.
24. Ramirez-Santos J, Collado-Vides J, Garcia-Varela M, Gomez-Eichelmann MC, Conserved regulatory elements of the promoter sequence of the gene rpoH of enteric bacteria, *Nucleic Acids Res* **29**:380–386, 2001.
25. Rodionov DA, Gelfand MS, Hugouvieux-Cotte-Pattat N, Comparative genomics of the KdgR regulon in Erwinia chrysanthemi 3937 and other gamma-proteobacteria, *Microbiology* **150**:3571–3590, 2004.
26. Yellaboina S, Ranjan S, Chakhaiyar P, Hasnain SE, Ranjan A, Prediction of DtxR regulon: identification of binding sites and operons controlled by Diphtheria toxin repressor in Corynebacterium diphtheriae, *BMC Microbiol* **4**, 2004.
27. Erill I, Jara M, Salvador N, Escribano M, Campoy S, Barbe J, Differences in LexA regulon structure among Proteobacteria through in vivo assisted comparative genomics, *Nucleic Acids Res* **32**:6617–6626, 2004.
28. Florea L, McClelland M, Riemer C, Schwartz S, Miller W, EnteriX 2003: Visualization tools for genome alignments of Enterobacteriaceae, *Nucleic Acids Res* **31**:3527–3532, 2003.
29. Yellaboina S, Seshadri J, Kumar MS, Ranjan A, PredictRegulon: a web server for the prediction of the regulatory protein binding sites and operons in prokaryote genomes, *Nucleic Acids Res* **32**(Web Server issue):W318–320, 2004.
30. Begley TP, Kinsland C, Mehl RA, Osterman A, Dorrestein P, The biosynthesis of nicotinamide adenine dinucleotides in bacteria, *Vitam Horm* **61**:103–119, 2001.
31. Kurnasov OV, Polanuyer BM, Ananta S, Sloutsky R, Tam A, Gerdes SY, Osterman AL, Ribosylnicotinamide kinase domain of NadR protein: identification and implications in NAD biosynthesis, *J Bacteriol* **184**:6906–6917, 2003.
32. Penfound T, Foster JW, NAD-dependent DNA-binding activity of the bifunctional NadR regulator of Salmonella typhimurium, *J Bacteriol* **181**:648–655, 1999.
33. Foster JW, Park YK, Penfound T, Fenger T, Spector MP, Regulation of NAD metabolism in Salmonella typhimurium: molecular sequence analysis of the bifunctional nadR regulator and the nadA-pnuC operon, *J Bacteriol* **172**:4187–4196, 1990.

34. Blattner FR *et al.*, The complete genome sequence of Escherichia coli K-12, *Science* **277**:1453–1474, 1997.
35. Wei J *et al.*, Complete genome sequence and comparative genomics of Shigella flexneri serotype 2a strain 2457T, *Infect Immun* **71**:2775–2786, 2003.
36. Parkhill J *et al.*, Complete genome sequence of a multiple drug resistant Salmonella enterica serovar Typhi CT18, *Nature* **413**:848–852, 2001.
37. Bell KS *et al.*, Genome sequence of the enterobacterial phytopathogen Erwinia carotovora subsp. atroseptica and characterization of virulence factors, *Proc Natl Acad Sci U S A* **101**: 11105–11110, 2004.
38. Parkhill J *et al.*, Genome sequence of Yersinia pestis, the causative agent of plague, *Nature* **413**:523–527, 2001.
39. Duchaud E *et al.*, The genome sequence of the entomopathogenic bacterium Photorhabdus luminescens, *Nat Biotechnol* **21**:1307–1313, 2003.
40. Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL, GenBank, *Nucleic Acids Res* **31**:23–27, 2003.
41. Schneider TD, Stephens RM, Sequence Logos: A New Way to Display Consensus Sequences, *Nucleic Acids Res* **18**: 6097–6100, 1990.
42. Tatusov RL, Galperin MY, Natale DA, Koonin EV, The COG database: a tool for genome-scale analysis of protein functions and evolution, *Nucleic Acids Res* **28**:33–36, 2000.
43. Felsenstein J, Evolutionary trees from DNA sequences: a maximum likelihood approach, *J Mol Evo* **17**:368–376, 1981.
44. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG, The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools, *Nucleic Acids Res* **24**:4876–4882, 1997.
45. Mironov AA, Vinokurova NP, Gel'fand MS, Software for analyzing bacterial genomes, *Mol Biol (Mosk)* **34**:253–262, 2000.
46. Friedberg D, Midkiff M, Calvo JM, Global versus local regulatory roles for Lrp-related proteins: Haemophilus influenzae as a case study, *J Bacteriol* **183**:4004–4011, 2001.
47. Gelfand MS, Laikova ON, in *Frontiers in Computational Genomics*, Michael YG and Eugene VKC (eds.), Academic Press, Wymondham, UK, pp. 203–204, 2003.

Anna V. Gerasimova is a graduate student in the Laboratory of Bioinformatics, State Scientific Center GosNIIGenetika, Moscow, Russia. She obtained her M.S. in Biophysics from Moscow Engineering Physics Institute (Technical University) in 2002. Her research interests are in comparative genomics and molecular evolution.

Mikhail S. Gelfand is the Head of the Research and Training Center in Bioinformatics of the Institute for Information Transmission Problems, RAS in Moscow, Russia and a Professor at the Department of Bioengineering and Bioinformatics of the Moscow State University. He graduated from the Department of Mathematics of the Moscow State University, received his Ph.D.

(Math.) degree from the Institute of Theoretical and Experimental Biophysics, RAS (Pushchino), and the Doctor of Sciences degree from the State Research Institute for Genetics and Selection of Industrial Microorganisms (Moscow). He is a member of editorial boards of several journals, in particular, "PLoS Biology",

"Bioinformatics", "BMC Bioinformatics", "Journal of Bioinformatics and Computational Biology" and "Journal of Computational Biology". He received the A. A. Baev prize (1999) from the Russian State "Human Genome" Council, and "The Best Scientist of the Russian Academy of Sciences" award (2004). His research interests include comparative genomics, metabolic reconstruction and modeling, evolution of metabolic pathways and regulatory systems, function and evolution of alternative splicing, functional annotation of genes and regulatory signals.