# Subsystem: Transport of Nickel and Cobalt

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Transition metals Nickel and Cobalt are essential components of many metalloenzymes [1]. Ni-dependent enzymes are urease, [NiFe] hydrogenase, [Ni] superoxide dismutase, CO dehydrogenase, and methyl-CoM reductase. In the form of coenzyme B12, cobalt plays a number of crucial roles in many biological functions. Also, there are some noncorrin-cobalt-containing enzymes (e.g. nitrile hydratase). Synthesis of Ni / Co enzymes and coenzyme B12 requires high-affinity uptake of the metal ions from natural environments where they are available only in trace amounts. Ni and Co uptake in bacteria is mediated by various secondary transporters and by at least two different ATP-binding cassette (ABC) systems [2,3]:

• Secondary transporters from the NiCoT family are able to uptake either both Ni and Co, or prefer only Ni ions [4]. NiCoTs are widespread among bacteria and found in some archaea and fungi. Substrate preferences correlate with the genomic localization of NiCoT genes adjacent to clusters of Ni/Co -dependent enzymes and enzymes of B12 biosynthesis, as well as with the presence of Ni or B12 regulatory sites upstream.

• Secondary transporters from the UreH family are Ni-specific and are often clustered with either urease or [Ni] supeoxide dismutase.

• Secondary transporters from the HupE/UreJ family are widespread among bacteria and encoded within certain [NiFe] hydrogenase and urease gene clusters. Most of them are Ni-specific transporters, however, in cyanobacteria the *hupE* orthologs appear to be under control of B12 riboswitch, and thus are ascribed to be Co-specific.

• High affinity Ni-specific ABC transporter NikABCDE is present in many proteobacteria and is regulated by NikR. NikA is a periplasmic substrate-binding component, NikB and NikC are permease components, and NikD and NikE are ATPases. Since NikABCDE systems belong to the nickel/peptide/opine PepT family, it is quite difficult to annotate their homologs in species distantly related to proteobacteria. Analysis of regulatory elements (NikR sites or B12 riboswitches) is useful in predicting Ni and Co substrate specificities. Diverged branches of Ni-specific systems (Nik-2, Nik-3) were detected in methanogenic archaea and some proteobacteria.

• Another Ni/Co ABC system, consisting of four to five components was identified based on genome context analysis. It consists of three conserved components (integral membrane proteins CbiM/NikM and CbiQ/NikQ; and ATPase CbiO/NikO). The Co-specific ABC systems contain a small component (CbiN) with 2 transmembrane segments and a short peptide loop between them, which could be involved in substrate recognition in place of a classical substrate-binding component of ABC transporters, missing in all CbiMNQO transporters. The Ni-specific ABC systems contain either the NikN or NikL additional component with topology similar to that of CbiN. However, they are not similar to CbiN on the sequence level. In many genomes NikM and NikN orthologs are fused into a single protein. In some species NikLMQO cassette is accompanied by a gene encoding putative periplasmic protein NikK, which can potentially serve as a Ni-binding component of an ABC transporter [5].

• CbtA and CbtC are the two novel B12-regulated secondary transporters for Co that were predicted based on comparative genome analysis [6].

Screening for B12-specific regulatory elements (*B12* riboswitches) or nickel repressor (NikR) binding sites within an upstream region of a gene accompanied by analysis of it's co-localization with B12 biosynthetic genes or ORFs encoding Ni-dependent enzymes - are powerful tools that can be applied to predict substrate specificities of a large number of candidate Ni and Co transporters and to identify new types of Ni/Co transporters [5, 6].

Fig. 1. Uptake of Nickel and Cobalt across cytoplasmic and outer membranes
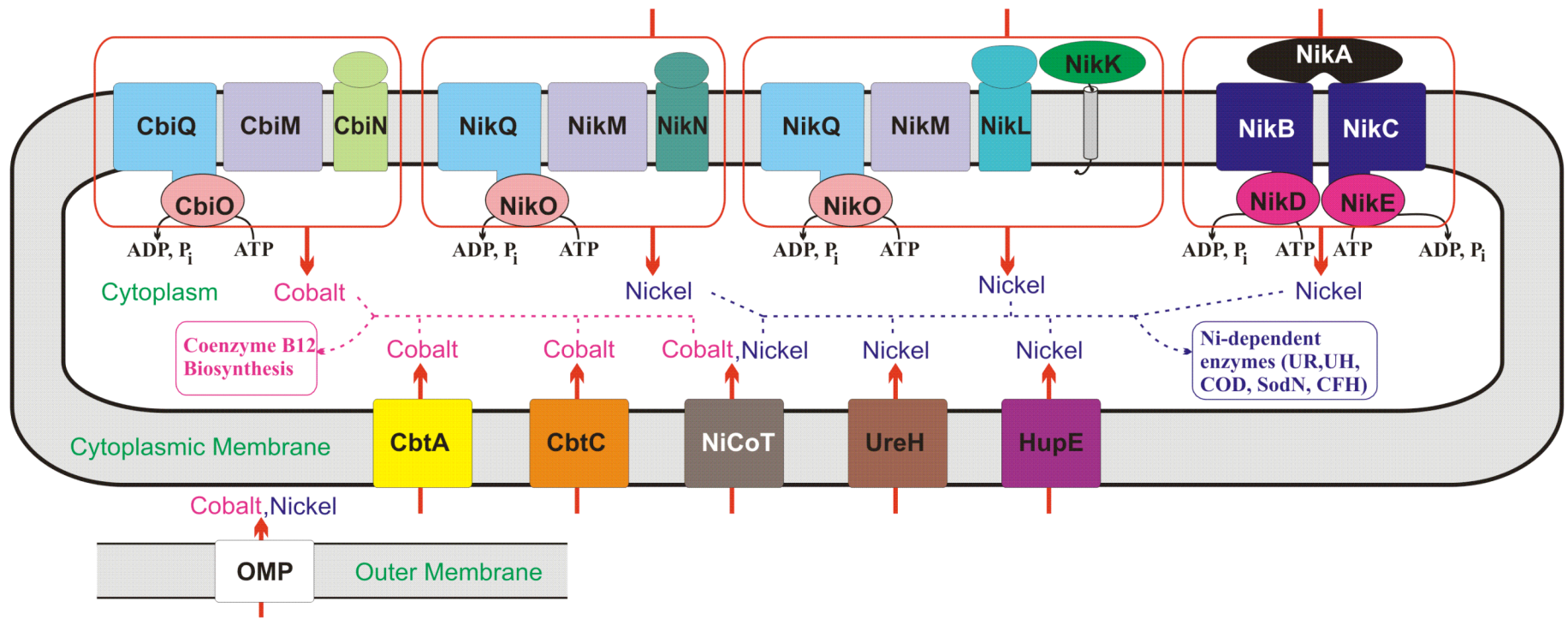
#	Abbrev.	Functional Role
1	OMP	Nicel/Cobalt-specific TonB-dependent outer membrane receptor
2	NiCoT	HoxN/HupN/NixA family nickel/cobalt transporter
3	HupE	HupE-UreJ family metal transporter
4	UreH	Nickel transporter UreH
5	CbtA	Predicted cobalt transporter CbtA
6	CbtC	Predicted cobalt transporter CbtC
7	NikK	Nickel ABC transporter periplasmic component NikK
8	NikL	Nickel ABC transporter component NikL
9	NikM	Nickel ABC transporter component NikM
10	NikN	Nickel ABC transporter component NikN
11	NikQ	Nickel ABC transporter permease component NikQ
12	NikO	Nickel ABC transporter ATPase component NikO
13	CbiM	Cobalt ABC transporter component CbiM
14	CbiN	Cobalt ABC transporter component CbiN
15	CbiQ	Cobalt ABC transporter permease component CbiQ
16	CbiO	Cobalt ABC transporter ATPase component CbiO

#	Abbrev.	Functional Role
17	NikA	Nickel ABC transporter, periplasmic nickel-binding protein nikA
18	NikB	Nickel transport system permease protein nikB (TC 3.A.1.5.3)
19	NikC	Nickel transport system permease protein nikC (TC 3.A.1.5.3)
20	NikD	Nickel transport ATP-binding protein nikD (TC 3.A.1.5.3)
21	NikE	Nickel transport ATP-binding protein nikE (TC 3.A.1.5.3)
22	NikA2	Nickel ABC transporter, periplasmic nickel-binding protein nikA2
23	NikB2	Nickel transport system permease protein nikB2 (TC 3.A.1.5.3)
24	NikC2	Nickel transport system permease protein nikC2 (TC 3.A.1.5.3)
25	NikD2	Nickel transport ATP-binding protein nikD2 (TC 3.A.1.5.3)
26	NikE2	Nickel transport ATP-binding protein nikE2 (TC 3.A.1.5.3)
27	UR	Urease alpha subunit (EC 3.5.1.5)
28	UH	Uptake hydrogenase large subunit (EC 1.12.99.6)
29	COD	Carbon monoxide dehydrogenase (EC 1.2.99.2)
30	SodN	Nickel-dependent superoxide dismutase (EC 1.15.1.1)
31	CFH	Coenzyme F420-reducing hydrogenase, gamma subunit

Fig. 2. Uptake of Nickel and Cobalt . Subsystem spreadsheet.

| Organism	Variant Code	NiCoT	HupE	UreH	CbtA	CbtC	NikK	*NiCoLN: NikL-8, NikN-10, CbiN-14	*NiCoM: NikM-9, CbiM-13	*NiCoQ: NikQ-11, CbiQ-15	*NiCoO: NikO-12, CbiO-16	*NikABCDE1: NikA-17, NikB-18, NikC-19, NikD-20, NikE-21	*NikABCDE2: NikA-22, NikB-23, NikC-24, NikD-25, NikE-26
Chlorobium tepidum TLS	1							391-14	392-13	390-15	389-16		
Lactobacillus plantarum WCFS1	2							542-10	543-9	541-11	540-12		
Haemophilus influenzae R2866	3						676	677-8	678-9	679-11	680-12		
Helicobacter pylori 26695	4	1064											
Aquifex aeolicus VF5	5		472										
Microbulbifer degradans 2-40	6			2484									
Escherichia coli K12	7											3409-17, 3410-18, 3411-19, 3412-20, 3413-21	
Yersinia pseudotuberculosis	7												2034-22, 2035-23, 2036-24, 1031-25, 1030-26
Polaromonas sp. JS666	8				5355								
Sinorhizobium meliloti 1021	9					5170							
Clostridium acetobutylicum ATCC 824	12							936-10, 1523-14	936-9, 1522-13	937-11, 1524-15	938-12, 1525-16		
Dechloromonas aromatica RCB	14	1983						1449-14	1450-13	1448-15	1447-16		
Salmonella typhimurium LT2	147	2685						1947-14	1948-13	1946-15	1945-16		1213-22, 1214-23, 1215-24, 1216-25, 1217-26
Synechocystis sp. PCC 6803	35		1137					2416-8	2415-9	2414-11	2413-12		
Synechococcus sp. WH 8102	56		2120	1623									
Pseudomonas fluorescens PfO-1	58		693		1878								

Functional variants:

#1: CbiMNQO: cobalt ABC transporter;

#2. NikMNQO: nickel ABC transporter;

#3: NikLMQO: nickel ABC transporter;

#4: NiCoT: secondary nickel/cobalt transporter;

#5: HupE: secondary nickel/cobalt transporter;

#6: UreH: secondary nickel transporter;

#7: NikABCDE or NikABCDE2: nickel ABC transporter;

#8: CbtA: predicted cobalt transporter (secondary);

#9: CbtC: predicted cobalt transporter (secondary).

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All other variant codes (two to three digits) are combination of the above nine (some organisms contains several nickel transporters or both nickel and cobalt transporters).
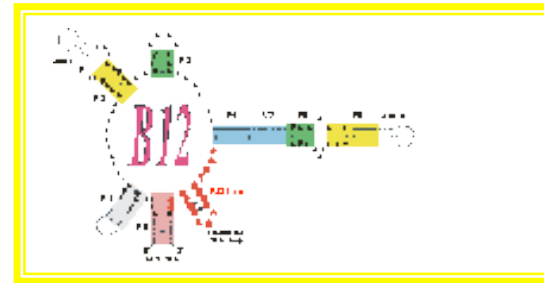
Fig. 3. Prediction of nickel and cobalt specificity of transporters [5, 6]
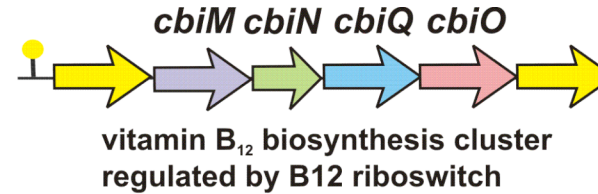
A. Analysis of regulatory elements

NikR operators (nickel repressor)
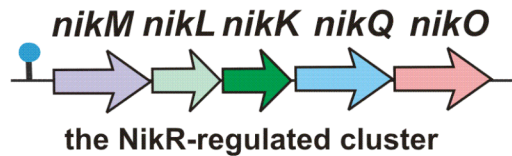- coregulates Ni transporters
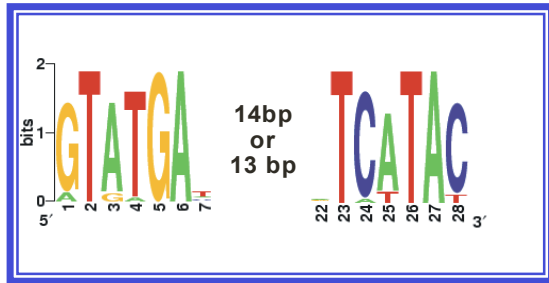
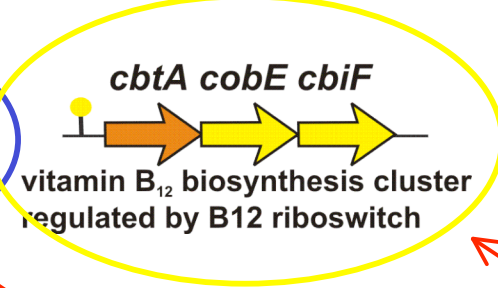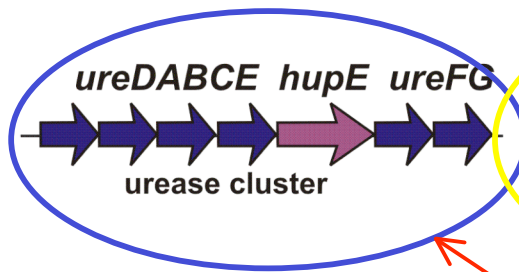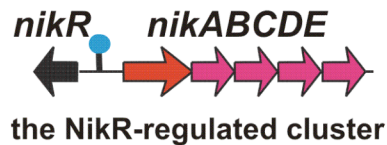**B12 riboswitch (RNA regulatory element)**
- coregulates Co transporters

B. Analysis of positional clustering with Ni-dependent enzymes or B12 biosynthesis genes

Fig. 4. The NiCoT family of Nickel/Cobalt transporters: mixed specificities [5]

Ta1169

RhRh_NhlF

Rhodococcus

TTP0007   *Thermus*

glr0533   *Cyanobacteria*   *Proteobacteria*

Lpg2477

Daro03000727

Reut0504

MT_nicT

MMR

SAV560

Krad060014

SAV2333

Actinobacteria

SSO1018

STok2443

Archaea   *Firmicutes*

Lmes1813
Lbrev
PpenA01000
SA2489

Helicobacter   HP_nixA
Hmu

BCE3656

Spo_Nic1p

MG05503

Q7S3L8_NEU

FG06513

Proteobacteria   *Fungi*

BPS
Bucepa02000285
RPA0724
RE_hoxN
Saro3914
Bcep2942
Bucepa02000753

RSp0628
CV1573
Bll6949:HupN
Bll6910
ERW
KP
ERC

Bcep7360

Bucepa02004503

Plu2170   YPO2673   YE

ST_nxiA

Regulation:
B12 riboswitch
NikR repressor

Synteny:
● B12 biosynthesis
● Ni-dependent enzymes
   (U, HD)

Exp.data about specificity:
Co>Ni ——
Ni>Co ——
Ni²⁺ ——
only Ni ——

References.

1. Mulrooney SB, Hausinger RP. Nickel uptake and utilization by microorganisms. *FEMS Microbiol Rev*. 2003; 27:239-61. *Review*.
2. Eitinger T, Mandrand-Berthelot MA. Nickel transport systems in microorganisms. *Arch Microbiol*. 2000; 173:1-9. *Review*.
3. Eitinger T, Suhr J, Moore L, Smith AC. Secondary Transporters for Nickel and Cobalt Ions: Theme and Variations. *Biometals*. 2005, *in press*.
4. Hebbeln P, Eitinger T. Heterologous production and characterization of bacterial nickel/cobalt permeases. *FEMS Microbiol Lett*. 2004, 230:129-35.
5. Rodionov DA, Hebbeln P, Maurel J, Gelfand MS, Eitinger T. Comparative genomic analysis of Nickel and Cobalt uptake transporters in bacteria. Characterization of a novel ABC-type transport system. *in preparation*.
6. Rodionov DA, Vitreschak AG, Mironov AA, Gelfand MS. Comparative genomics of the vitamin B12 metabolism and regulation in prokaryotes. *J Biol Chem*. 2003; 278:41148-59.