

ZitB Eco -----MAHSHSHTSSHLP-EDNNARRL 21
 ZitB Sty -----MAHSHSHADSHLP-KDNNARRL 21
 CzcD Bsu -----MGHNNHNEG-----ANKKVL 14
 CzrB Sau -----MSHSHHHDHMHSHVTTNNKKVL 22
 CzcD Rme -----MGAGHSHDHPGGNE-----RSL 17
 Znt2 Rno MASRSFFGALWKSEASRI PPNLPSVELAVQSNHYCHAQKDSGSHPNSEKQ---RARRKL 57
 Cot1 Sce -----MKLGSKQVKI 10
 Zrc1 Sce -----MITG-KELRI 9
 Znt1 Rno -----MGCWGRNRGRL 11
 Znt1 Mmu -----MGCWGRNRGRL 11
 Yiip Eco -----MN-QSYGRLVSRAA 13
 P34 Rre -----MDTNSRNRLIKSAS 14

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ZitB Eco LYAFGVTAGFMLV**EV**VGGFLSGSLALLADAG**HML**TDTAALLFALLAVQFSR-RPPTIRHT 80
 ZitB Sty LFAFIVTAGFM**LE**VVGGILSGSLALLADAG**HML**TDAAALLFALLVVQFSR-RPPTVRHT 80
 CzcD Bsu LISFIMITGYMI**IE**AIGGFLTNSLALLSDAG**HML**SDSISLMVALIAFTLAE-KKANHNKT 73
 CzrB Sau FISFLIIGLYMFI**EI**IGGLANSALALLSDGI**HMF**SDTFSLGVAFVAFIYAE-KNATTTKT 81
 CzcD Rme KIALALTGTF**LIA**EVVGGVMTKSLALISDA**AHML**TDTVALAIALAAIAIAK-RPADKKRT 76
 Znt2 Rno YVASAICLVFMIG**EI**IGGYLAQSLAIMTDA**HL**LLTDFASMLISLFSLWVSS-RPATKTMN 116
 Cot1 Sce ISLLLLDTVFFGI**EI**ITGYLSHSLALIADS**FHML**NDIISLVVALWAVNVAKNRNPDSYTY 70
 Zrc1 Sce ISLLTLDTVFF**LLE**ITIGYMSHSLALIADS**FHML**NDIISLLVALWAVDVAKNRGPDAKYT 69
 Znt1 Rno LCMLLLTFMFMV**LE**VVSRVTASLAMLSDS**FHML**SDVLALVVALVAERFARRTHATQKNT 71
 Znt1 Mmu LCM**LL**TFMFMV**LE**VVSRVTASLAMLSDS**FHML**SDVLALVVALVAERFARRTHATQKNT 71
 Yiip Eco IAATAMAS**LL**LLIK**IF**AWWYTGVS**SILAALVDS**LVDIGASLTNLLVVRYSL-QPADNHS 72
 P34 Rre YLSVTTALII**LSIK**LYAWVVTDSQS**ILAALIDS**MLDITSSFINLIALRFAL-QPPDHHR 73

ZitB Eco FGWLRLTTLAAFVNAIALVVITILIVWEAIERFRT-PRPVEGMMMAIAVAGLLANILSF 139
 ZitB Sty FGWLRLTTLAAFVNAIALVVITLLIVWEAIERFYT-PRPVAGNLMMAIAVAGLLANLFAF 139
 CzcD Bsu FGyKRFEILAAVINGAALILISLYIYEAIERFSN-PPKVATGMLTISIIGLVNLLVA 132
 CzrB Sau FGyKRFEVLAALFNGVTLFVVISILIVFEAIKRFFV-PSEVQSKEMLIISIIGLIVNIVVA 140
 CzcD Rme FGYYRFEILAAAFNALLFGVAIYIILEAYLRKLS-PPQIESTGMFVAVLGLIINLIS- 134
 Znt2 Rno FGWQRAEILGALLSVLSIWVVTGVLVYAVQRLISGDYEIKGDTMLITSGCAVAVNIIMG 176
 Cot1 Sce YGWKRAEILGALINAVFLIALCVSILIEALQRIIAPPVIENPKFVLVYGVAGLISNTVGL 130
 Zrc1 Sce YGWKRAEILGALINAVFLIALCFSIMIEALQRLIEPQEIQNRLVLYVGVAGLISNVVGL 129
 Znt1 Rno FGWIRAEVVMGALVNAIFLTGLCFAILLEAVERFIEPHEMQQPLVVLVSVGVAGLLVNLGL 131
 Znt1 Mmu FGWIRAEVVMGALVNAIFLTGLCFAILLEAVERFIEPHEMQQPLVVLVSVGVAGLLVNLGL 131
 Yiip Eco FGHGKAESLAALAQSMFISGSALFLFTGIQHLSPTPMTDPGVGVIVTIVALICTIILV 132
 P34 Rre FGYEKLQDLTIFSQSIFFFASAFFVGFSSVKSLEKTKPENISDGTVMYVICFLTIIIV 133

ZitB Eco WLLHH----- 144
 ZitB Sty WILHR----- 144
 CzcD Bsu WIMMS----- 137
 CzrB Sau FFMFK----- 145
 CzcD Rme ----- 145
 Znt2 Rno LALHQS----- 182
 Cot1 Sce FLFHDN-DQEHGHGHSHGGIFA-DHEMMPSSHTHTHAHVDGIENTTPMDSTDNISEI 188
 Zrc1 Sce FLFHDH-GSDS--LHSHSHGSVESGNNLDIESNATHSHSHAS-LPNDNLAIDEDAISSP 185
 Znt1 Rno CLFHHHSGEGQAGAGHSHGHGHGLAKGARKAGRAGGEAGAPPGRAPDQEPDQEEETNTL 191
 Znt1 Mmu CLFHHHSGEGQAGAGHSHGHGHGLAKGARKAGRAGVEAGAPPGRAPDQ---EEETNTL 187
 Yiip Eco SFQRW----- 137
 P34 Rre FYQTY----- 138

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ZitB Eco -----GSEK-NLNVRAAALHV 160
 ZitB Sty -----GSDEK-NLNVRAAALHV 160
 CzcD Bsu -----GGDTKNNLNIRGAYLHV 154
 CzrB Sau -----GGDTSHNLMNRGAFLHV 162
 CzcD Rme -----MRMLSSGQSSSLNVKGAYLEV 155
 Znt2 Rno -----GHGSHGHSHEDSSQQQNPVRAAFHV 211
 Cot1 Sce MPNAIVDSFMNENTRLLTPENASKTPSYSTSSHTIASGGNYTEHNKRKRSLNMHGVFLHV 248
 Zrc1 Sce GPSPQIGEVLPQSVVNRLSNEQPLLNHDDHDSHES-----KKPGHRSLNMHGVFLHV 239
 Znt1 Rno VANTSNSNGLKADQAEPEKLRSDDPVDVQVNGNLIQESDSLESEDNRAGQLNMRGVFLHV 251
 Znt1 Mmu VANTSNSNGLKADQAEPEKLRSDDPVDVQVNGNLIQESDNLEAEDNRAGQLNMRGVFLHV 247
 Yiip Eco -----VVRTQSQAVRADMLHY 154

P34 Rre -----VIKKTGSEIVKADK~~LY~~ 155

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ZitB Eco LGDLLGSVGAIIAALII IWT-----G 181
ZitB Sty MGDLLGSVGAIVAALII IWT-----G 181
CzcD Bsu ISDMLGSVGAIIAALII IFF-----G 175
CzrB Sau TGDLLGSVGAITAAALI IWF-----G 183
CzcD Rme WSDLLGSVGVIAGAI IIRFT-----G 176
Znt2 Rno VGDLLQSVGVLVAAAYII YFKP-----E 233
Cot1 Sce LGDALGNIGVMLS AFFIWK-----TDYSW 272
Zrc1 Sce LGDALGNIGVIAAALFIWK-----TEYSW 263
Znt1 Rno LGDALGSVIVVVALVFYFSWKGCTEDDFCVNPFDPCKSSVELMNSTQAPMHEAGPCW 311
Znt1 Mmu LGDALGSVIVVVALVFYFNWKGCTEDDFCTNPFDPCKSSVEIINSTQAPMRDAGPCW 307
Yiip Eco QSDVMMNGAILLALGLSWY-----G 174
P34 Rre FTDLLTNVIVII SINLSDY-----F 175

6 **7** **8** **910**

ZitB Eco WTPADPILSILVSLVLRSAWRLLKDSVNELEGAPVSLDIAELKRRMCREIPEVRNV~~HH~~ 241
ZitB Sty WTPADPILSILVSVLVRSAWRLLKDSVNELEGAPVSLDINALQRHLSREIPEVRNV~~HH~~ 241
CzcD Bsu WGWADPLASII VAILVLRSGYNVTKDSIHILMEGTPENIDVSDIIRTIEGTE-GIQNI~~HD~~ 234
CzrB Sau WTIADPIASILVSVIILKSAWGITKSSINILMEGTPSDVDIDEVITTIKKS-RIQSV~~HD~~ 242
CzcD Rme WAWVDSIAI AVLIGLWVLPRTWILLKSSLNVLEGVPPDDVLA EVEKQILATP-GVKS~~FHD~~ 235
Znt2 Rno YKYVDPICTFLFSILVLTTLTILRDVILVLMEGTPKGVDFTTVKNLLSVD-GVEAL~~HS~~ 292
Cot1 Sce KYYTDPVLSLIITGII FSSALPLSCKASKILLQATPSTLSGDQVEGDLKIP-GIIAI~~HD~~ 331
Zrc1 Sce RYSDPIVSLIITIIIFSSALPLSRRASRILLQATPSTISADQIQREILAVP-GVIAV~~HD~~ 322
Znt1 Rno VLYLDP~~TL~~CIIMVCILYTTYPLLKESALILQLTVPKQIDIKHLVKELRDVE-GVEEV~~HE~~ 370
Znt1 Mmu VLYLDP~~TL~~CIIMVCILYTTYPLLKESALILQLTVPKQIDIKHLVKELRDVD-GVEEV~~HE~~ 366
Yiip Eco WHRADALFALGIGIYILYSALRMGYEAVQSLDRALPDEERQEIIIDIVTSWP-GVSGA~~HD~~ 233
P34 Rre WF-VDP~~LF~~GVVISLYIFHSSYSLFKKA~~FN~~LVDELPEQDRQKIIISIVNNHL-GAKGM~~HE~~ 233

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ZitB Eco VHVWVMVG-EKPVMTLHVQV-----IPPHDHDALLDQIQHYLMDHYQIEHATIOMEYQ~~CH~~ 295
ZitB Sty VHVWVMVG-EKPVMTLHAQV-----IPPHDHDALLERIQDFLMHEYHIAHATIOMEYQ~~MCH~~ 295
CzcD Bsu LHIWSTSGLNALSCHAVVDD--QLTISESENILRRIEHEHELEHKG-ITHVTIQMETEA~~HN~~ 291
CzrB Sau CHVWTISNDMNALSCHVVVDH--TLTMKECELLLENIEHDLHLN-IHMTIQLET~~PNHK~~ 299
CzcD Rme LHIWALTSGKASLTVHVVN---DTAVNPEMEVLP~~EL~~KQMLADKFDITHVTIQFELAP~~CE~~ 291
Znt2 Rno LHIWALTVAQPVLSVHIAI---AQNVDAQAVLKVARDRLQ~~GK~~FNFTMTIQIESY~~ED~~ 347
Cot1 Sce FHIWNLTESIFIASLHIQLDISPEQFTDLAKIVRSKLRHYGIHSATLQPEFITREV--- 387
Zrc1 Sce FHVWNLTESIYIASIHVQIDCAPDKFMSSAKLIRKIFHQHGIHSATVQPEFVSGDVNEDI 382
Znt1 Rno LHVWQLAGSRIIATAHIKCED-PASYMQVAKTIKDVFNHNGIHATTIQPEFASVSGSKS~~SV~~ 429
Znt1 Mmu LHVWQLAGSRIIATAHIKCED-PASYMQVAKTIKDVFNHNGIHATTIQPEFASVSGSKS~~SV~~ 425
Yiip Eco LRTRQSG-PTRFIQIHLEMED--SLPLVQAHMVADQVEQAILRRFP~~GS~~DVIIHQD~~PCS~~VV 290
P34 Rre MKTRYAG-QKAFIQCHLEMDG--NMSLYNAHKISDEIAFEILQEFPEAEI I IHQD~~PP~~GI 290

ZitB Eco GP-----DCHLNEGVS~~GH~~SHHH----- 313
ZitB Sty GP-----DCHLNQTPSGHV~~HHH~~----- 312
CzcD Bsu HD-----NAILCQPKMEKQRD~~HHH~~----- 311
CzrB Sau HD-----ESIICSGTHSHSHNH~~HHH~~HAHVH----- 325
CzcD Rme QA-----DAAQHFNASPALVGSKSLAAGGN----- 316
Znt2 Rno MK-----SCQECQGPSE----- 359
Cot1 Sce -----TSTERAGDSQGDHLQNDPLSLRPKTYGTGISGSTCLIDDAANCNTADC 435
Zrc1 Sce RRRFSIIAGGSPSSQEA~~FD~~SHG-NTEHGRKKRSPTAYGATTASSNCIVDDAVNCNTSNC 441
Znt1 Rno VP--CELACRTQCALKQCCGTRPQVHSGKEAEKAPT~~VS~~ISCLELSENLEKKPRRTKAEGS 487
Znt1 Mmu LP--CELACRTQCALKQCCGTRPQVHSGKDAEKAPT~~VS~~ISCLELSENLEKKARRTKAEGS 483
Yiip Eco PR-----EGKRSMLS----- 300
P34 Rre EH-----VKYREYIVR----- 301

ZitB Eco -----
ZitB Sty -----
CzcD Bsu -----
CzrB Sau -----
CzcD Rme -----
Znt2 Rno -----
Cot1 Sce LEDH----- 439
Zrc1 Sce L----- 442
Znt1 Rno VPAVVIEIKNV~~PN~~KQPESSL 507
Znt1 Mmu LPAVVIEIKNV~~PN~~KQPESSL 503
Yiip Eco -----
P34 Rre -----

Figure 1: Multiple alignment of the CDF family proteins. The residue # in each protein is indicated by the number presented at each end of the line. Mutated residues in bold print are indicated by the number on top 1: E35, 2: H53, 3: M54, 4: H159, 5: D163, 6: D186, 7: E214, 8: D221, 9: H240, 10: H241, 11: G248, and 12: C294). The accession number of each protein is as follows. ZitB *Escherichia coli*: P75757, CzcD *Ralstonia metallidurans*: CAA67085, YiiP *Escherichia Coli*: P32159, P34 *Rickettsia rickettsii*: P21559, Cot1 *Saccharomyces cerevisiae*: P32798, Zrc1 *Saccharomyces cerevisiae*: P20107, Znt1 *Rattus norvegicus*: Q62720, Znt1 *Mus mucus*: Q60738, Znt2 *Rattus norvegicus*: Q62941, CzcD *Bacillus subtilis*: C69612, CzcB *Staphylococcus aureus*: BAB43232, ZitB *Salmonella typhi*: Q8Z8B6.