Supporting information

Structural basis for cold adaptation.

What is the structural basis for the cold adaptation of the ATPase and unwinding activities of the psychrophilic SrmB and RhlE, respectively? Presumably, these adaptations do not reflect single, but multiple aminoacid substitutions (*1*). Compared with SrmB_Ec, SrmB_Ph might channel more energy from ATP and/or RNA binding, or from Pi release, into the rate-limiting steps of the ATPase cycle, *i.e.*into the 1->2 and 3->4 transitions (Fig. 7A). Thereby, these steps would be facilitated. Interestingly in this respect, alignment of the conserved Q-motif region suggests that SrmB_Ec and SrmB_Ph (but not the RhlE orthologs) differ in the precise way they interact with ATP (Fig. S2, A). However, sequence differences outside the conserved motifs (cf. Fig. 2) that are not directly related to substrate or product binding, might also contribute to the different behaviour of SrmB_Ph and SrmB_Ec. Indeed, starting from a mutant of the Ded1 protein apparently impaired in the 1->2 transition, Banroques et al. identified suppressor mutations that are widespread throughout the outer shell of the DEAD-box core (*2*).

Similarly, the reason why psychrophilic RhlEs might unwind more base pairs than RhlE_Ec in the closed conformation (Fig. 7C) remains elusive. Two residues that appear to contact RNA near the bent thought to be important for unwinding (*3*) differ between RhlE_Ec and the psychrophilic RhlEs (Fig. S2, B): an intriguing possibility, then, is that these differences participate to the unequal unwinding activities of these enzymes. Further work is needed to test these hypothesis.

Table S1.

Primers used in this study[a]

SrmB_Ph F	5’-CATG*CCATGG*AACAATTTTCTGAATTTGATCTTGATAAC
SrmB_Ph R	5’-GC*TCTAGA*TTACTTTTTCTTTTTAACCTTAGCTTTTTTC
SrmB_Cp F	5’-TCAG*GGCGCC*ATGTTTGAGCAATTCGATCTAGAC
SrmB_Cp R	5’-GGC*CTCGAG*GGGTATAACTTGGTAAAGGG
RhlE_Ph F	5’-TCGC*GGATCC*TTTGAAGGTTTAGGTTTATCACAGTC
RhlE_Ph R	5’-TAGCCG*CTCGAG*TTAATCGTTGTTACTTGGACGACG
RhlE_Cp F	5’-TCAG*GGCGCC*ATGAGTGATACCCCAACCAAG
RhlE_Cp R	5’-CCC*AAGCTT*TTTTTCTTTAAGGTGTTGAGCGTC
SrmB_Ph F1	5’-TTGAGCCTGCATGACTGAGC
SrmB_Ph R1	5’-GGCTTATTACTAAGCTTGGGC
SrmB_Cp F1	5’-CG*GAATTC*CCGATGAGCGCTTAGATACTTGG
SrmB_Cp R1	5’-CG*GAATTC*AAAAAAAAGCCCGCTCATTAGGCGGGCTGC TTGGGTATAACTTGGTAAAGGG

[a]Restriction sites used for cloning are italicized.

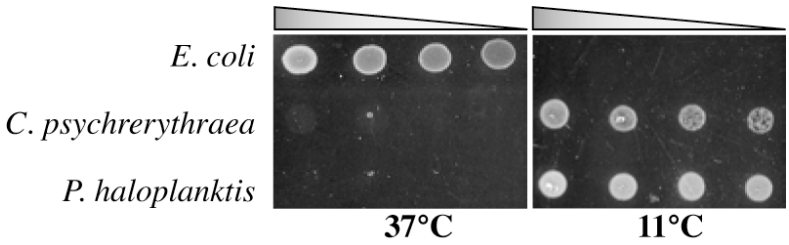

Figure S1. *P. haloplanktis* and *C. psycherythraea* are cold-adapted organisms. Whereas *E. coli* grows readily at 37°C (left) but not at 11°C (right), the reverse is true for its two psychrophilic relatives. Serial dilutions of saturated cultures were incubated for 24 h (37°C) or 48 h (11°C) on Difco Marine plates.

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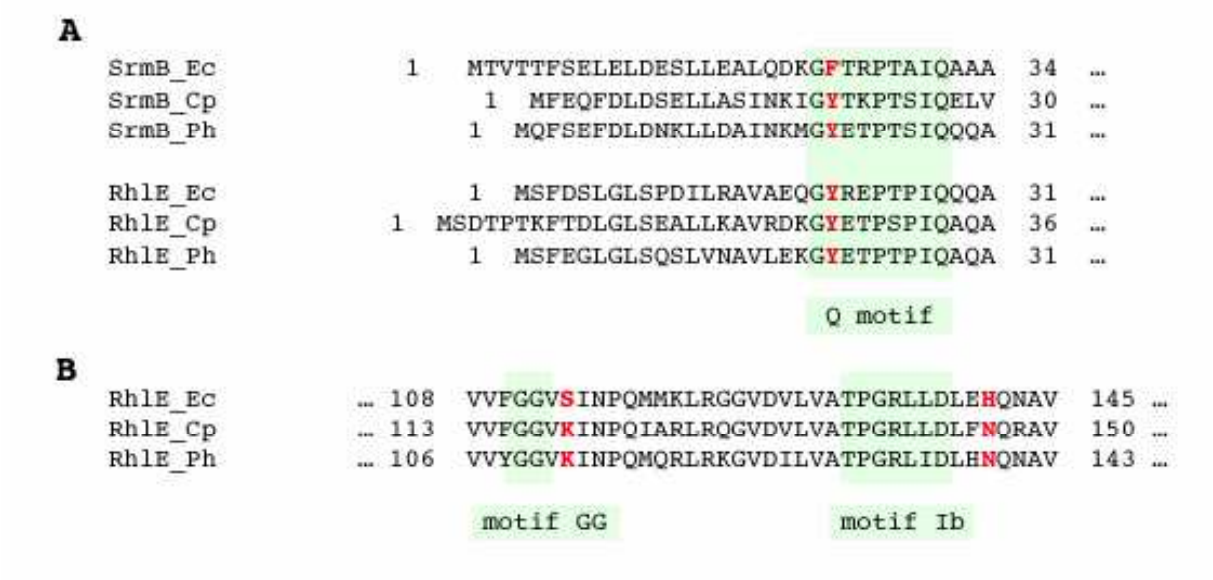

Figure S2. Possible correlation between sequence and cold-adaptation in the SrmB and RhlE series. (A) Alignment of the three SrmB orthologs (top) and of the three RhlE orthologs (bottom) in the region of the Q-motif (green box), which is involved in the recognition of the adenine moiety of ATP. Modelisation of RhlE_Ec and SrmB_Ec with the Geno3D software (geno3d-pbil.ibcp.fr) using the structure of Vasa (*3*) indicates that the aromatic residue noted in red stacks on the adenine ring. This residue differs for SrmB_Ec and SrmB_Ph, whose ATPase activity is differentially affected by temperature, but not for the three RhlE orthologs, whose ATPase is evenly affected by temperature. (B) Alignment of the three RhlE orthologs in the region of the GG and Ib motifs. Shown in red are two residues that, in the modelized structures of RhlE_Ec, are predicted to contact the bound RNA near a sharp bent thought to be the cause for unwinding (*3*). Equivalent contacts have actually been observed in the structures of eIF4AIII and Mss116 (*4, 5*). Note that these residues differ between RhlE_Ec and the cold-adapted RhlE_Ph and RhlE_Cp.

References

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