# **Supplementary material**

### Results

#### MJ1130 is an iron metalloprotein

The purified MJ1130 protein exhibits a pink color in solution resulting from a broad absorbance band centered at 492 nm as observed for the *Pyrococcus abyssi* ortholog PaKael (Hecker, et al., 2007) (Figure S1A). This broad absorbance corresponds to a charge-transfer transition from a tyrosinate to a Fe(III) ion suggesting that MJ1130 contains a Fe<sup>3+</sup> ion. This was confirmed by the EPR (electron paramagnetic resonance spectroscopy) spectrum obtained at pH 8.0 (Figure S1B) which exhibits features characteristic of a high-spin (S=5/2) Fe(III) monomer. Compared to the spectrum recorded on PaKae1 (Hecker, et al., 2007), the features are sharper and the low field signals have a smaller amplitude relative to the sharp g=4 signal. The broad signals (g=8.2 and 5.4) for PaKae1 had been interpreted as a mixture of species or at least conformational heterogeneity at the level of the iron site. In the case of MJ1130, the site seems to be more homogeneous and it exhibits lower rhombicity. Thus, MJ1130 also contains a Fe<sup>3+</sup> ion and the sharper signal indicates a lower conformational heterogeneity of the site compared to that of PaKae1.

#### The autophosphorylation activity of MjBud32 is inhibited by Mj-Kae1

We tested the kinase activity of the archaeal MJ1130 protein and of the MjKae1 and MjBud32 subdomains that we expressed independently in *E. coli*. The fusion protein, as well as the Bud32 moiety did not exhibit kinase activity on casein in our experimental conditions. However, both the fusion protein and the two subdomains exhibited an autophosphorylation activity (previously observed in the case of PaKae1, Hecker et al., 2007). These autophosphorylation activities were stronger when the proteins were tested separately (Figure S5). Furthermore, the autophosphorylation of MjBud32 was completely suppressed by the addition of the Kae1 moiety. Taken together our results indicate that archaeal Bud32 phosphotransferase activity is inhibited by Kae1. This fits well with the fact that we could not detect AMPPNP in the nucleotide binding site of MjBud32, suggesting that the presence of Kae1 maintains Bud32 in an inactive conformation both in the crystal and in solution.

# Material and methods

#### **Amplification and cloning**

#### Wild-type and mutant constructs of archaeal Mj1130, MjKae1 and MjBud32

The sequence encoding archaeal Mj1130, MjKae1 and MjBud32 (Kae1 and Bud32 domains of MJ1130 respectively) proteins were amplified by polymerase chain reaction using genomic DNA of *Methanocaldococcus jannaschii* strain DSM 2661 as template and specific primers containing a *Nde*I or a six histidine-tag fused to a *Not*I site as forward and reverse specific primers respectively. The PCR products were cloned into pGEM-T easy vector (Promega), then digested by appropriate restriction enzymes and finally subcloned in a modified pET-9a expression vector (Novagen) between *Nde*I and *Not*I restriction sites.

#### Wild-type and mutant constructs of yeast kae1 and bud32-his

Yeast Kae1p and Bud32p-his were coexpressed in *E. coli* in a polycistronic vector (namely 6pK-Sc-Ka,Bu-a), derived from pET28 vector (Novagen). The *kae1* gene was amplified by PCR and ligated at the *Not*I restriction site followed by the *Sc-bud32-his* gene which has a 6His-tag encoding sequence at the 3' end. *E. coli* expression with 6-pK-Sc-Ka,Bu-a leads to single transcripts bearing the two genes that both have their own rbs (ribosome binding site). Mutants of either *kae1* or *bud32* in the polycistron have been constructed with the QuikChange mutagenesis kit (Stratagene) by using 6-pK-Sc-Ka,Bu-a plasmid as template and and specific olignonucleotides (see Table S1) containing mutation.according to the supplier's instructions.

### Expression and purification of recombinant proteins

#### Expression and purification of archaeal Mj1130, MjKae1 and MjBud32 proteins

Expression of recombinant proteins were performed at 37°C using a transformed *E. coli* Rosetta (DE3) pLysS by the appropriate recombinant plasmid in LB medium containing kanamycin (50  $\mu$ g/ml) and chloramphenicol (34  $\mu$ g/ml). When the cell culture reached an OD<sub>600</sub> of 0.7, expression of recombinant protein was induced by 0.4 mM isopropyl  $\beta$ -D-thiogalactopyranoside (IPTG) for 4 hours. Cells were harvested by centrifugation,

resuspended in a Tris-HCl 20 mM pH 8.0, NaCl 200 mM (or 500 mM in the case of MjBud32), β-mercaptoethanol 5 mM buffer and stored overnight at -20°C. Cell lysis was completed by sonication. Cell extract was then centrifuged at 10,000 g for 15 min. at 4°C to remove cellular debris and aggregated proteins. His-tagged protein from soluble fraction was purified by gravity-flow chromatography on a nickel nitriloacetate agarose resin (Ni-NTA) column (Qiagen Inc.) according to the manufacturer's recommendations followed by a gel filtration step on a Superdex<sup>TM</sup> 75 column (GE Healthcare). The homogeneity of the protein was checked by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Protein concentration was determinate by measuring the absorbance at 280 nm using specific molar absorption coefficient.

#### Co-expression and co-purification of yeast Kae1p and Bud32p-his

Kae1p and Bud32p-his have been expressed by transforming *E. coli* Rosetta pLysS (Novagen) with 6-pK-Sc-Ka,Bu-a plasmid. Bacteria were grown at 37°C in 2xYT medium supplemented with kanamycin and chloramphenicol until the OD reached 0.6 to 0.8 and the growth temperature was then shifted to 15°C for one hour. Expression was then triggered by adding 0.5 mM of IPTG and the culture was left overnight at 15°C. Bacteria were harvested and stored at -20°C after being resuspended in Tris-HCl 20 mM pH 7.5, NaCl 200 mM, 2-mercaptoethanol 5 mM (lysis buffer). The same expression protocol has been used for the mutated proteins.

Co-purification of either wt or mutated Kae1p and Bud32p-his has been performed by IMAC (Immobilized Metal Affinity Chromatography). The bacteria were lyzed on ice by sonication and centrifuged at 20000 g for 25 min. The supernatant was recovered, precipitated with 0.2 % (final concentration) of polymin P, incubated on ice for 15 min. and centrifuged at 20000 g for 25 min. The resulting supernatant was mixed with Protino NiIDA resin (Macherey Nagel) for 30 min at 4°C and loaded onto a column. The resin was then washed with lysis buffer and the bound proteins were successively eluted with 3 fractions containing the same buffer supplemented with 100 mM, 200 mM and 400 mM imidazole. The two pellets obtained after the two centrifugation steps were also pooled in the same volume of lysis buffer used for the resuspension of bacteria and kept for subsequent analyses. All the fractions obtained after each purification step were analysed by SDS-PAGE or Western blotting.

#### Expression and purification of the yeast proteins used for the kinase assay

Bud32p-his, Bud32p-his D161A and Grx4p-his have been expressed in *E. coli* BL21 Gold (Stratagene) and purified by using the same pET20-derivative plasmids and same protocols as described by Lopreiato *et al.* (2004). Kae1p and Bud32p-his have been co-expressed and co-purified as already described above (see "*Co-expression and co-purification of yeast Kae1p and Bud32p-his*") except that we added a size exclusion chromatography step on Superdex<sup>TM</sup> 200 (GE Healthcare) just after elution from IMAC in order to isolate an equimolar ratio of Kae1p and Bud32p-his in the binary complex.

#### Phosphotransferase assay on archaeal proteins

The phosphotransferase activity of kinase was assayed on archaeal MJ1130, MjKae1 and MjBud32 proteins. MJ1130, MjBud32, MjKae1 (approx. 5  $\mu$ g) were incubated with 25  $\mu$ M [ $\gamma$ -<sup>32</sup>P]ATP (1  $\mu$ Ci/reaction, specific activity of 6000 Ci.mmol<sup>-1</sup>) in 20  $\mu$ l of buffer containing 50 mM Hepes-HCl pH 7.5, 100 mM NaCl, 1 mM DTT, 10 mM MnCl<sub>2</sub> for 30 min. at 72°C. After incubation, reactions were stopped by the addition of loading buffer and the samples analyzed by electrophoresis (SDS-PAGE). The gels were then socked in 16% TCA for 10 min. at 90°C and neutralized in 100 mM phosphate buffer pH 7.4. This procedure eliminates most unincorporated [<sup>32</sup>P], non-protein material, phosphorylation on His and Asp residues and enriches samples for *O*-phosphorylated proteins (Lévine *et al.*, 2006). The treated gels were finally stained in neutralization buffer supplemented with 5% TCA and 0.1% Coomassie blue and radiolabelled proteins visualized by autoradiography.

#### **Optical and spectroscopic characterization**

Visible spectra were recorded on a Cary 50 spectrophotometer (Varian Inc.) using 200  $\mu$ g or 100  $\mu$ g of recombinant MJ1130 or PaKae1 diluted in Tris-HCl 20 mM pH 8.0, NaCl 200 mM. EPR spectra were realized in the same conditions at 10 K on a Bruker Elexsys E500. Prior to measurements, the tubes were degassed with helium gas to remove oxygen from the samples.

#### **Co-Immunoprecipitation**

Extracts were prepared from wild type yeast cells, expressing an HA-tagged version of chromosomal Bud32p and ectopically expressing wt or mutant Kae1TAP from a pCM185 plasmid (the endogenous untagged version of Kae1p was not deleted in these strains). Yeast cells were first grown in selective minimal medium to minimize plasmid loss and transferred to 200 mL of YPDA medium for roughly two generations (OD600 1.0). Collected cells were broken in 1:1 w/v of AGK Buffer (Hepes 20 mM pH 7.9, MgCl2 1.5 mM, KCl 200 mM, Glycerol 10%, DTT 0.5 mM, AEBSF 1 mM, Complete antiprotease cocktail, Boehringer), extracts were cleared by two centrifugation steps (14000 rpm for 45 min and 54000 rpm for 30 min) and dialyzed against Buffer D (Hepes 20 mM pH 7.9, KCl 50 mM, EDTA 0.2 mM, Glycerol 20%, DTT 0.5 mM). Protein amounts have been determined by Bradford assay. TAP-tagged Kae1 proteins have been immunoprecipitated by incubating (3 hrs at 4°C) 3 mg of whole cell extracts with Sepharose IgG-beads affinity matrix (Amersham). Beads were washed 3 times with IPP150 Buffer (Tris-HCl 10 mM pH 8; NaCl 150 mM; NP40 0.1%) and bound proteins were eluted with 1X Laemmli for SDS-PAGE and Western blot analysis. Filters were probed with anti-HA antibody to reveal the presence of Bud32-HA in Kae1-TAP immunoprecipitates. Where indicated immunoprecipitations and washings were performed at different salt concentrations (50, 250, 500 mM NaCl).

#### Western Blotting

Rabbit polyclonal antibodies ( -Kae1p) against Kae1p have been raised by Genecust Company after providing them with Kae1p-his protein purified in denaturing condition. After SDS-PAGE, proteins were transferred onto a Hybond-P membrane (GE Healthcare) in Tris-HCl 25 mM pH 8.5, Glycin 192 mM, methanol 20%. The membrane was probed with  $\alpha$ -Kae1p (dilution 1:20000) for one hour in TBST (Tris-HCl 10 mM pH 8, NaCl 150 mM, Tween20 0.2%) then washed 3 times (10 minutes) in TBST and probed with the secondary anti-rabbit antibody (GE Healthcare) for one hour in TBST. After 3 wash steps the membrane was finally labelled with the ECL western blotting kit (GE Healthcare) according to the supplier's instructions.

# References

Hecker, A., Leulliot, N., Gadelle, D., Graille, M., Justome, A., Dorlet, P., Brochier, C., Quevillon-Cheruel, S., Cam, E. L., van Tilbeurgh, H. and Forterre, P. (2007). An archaeal orthologue of the universal protein Kae1 is an iron metalloprotein which exhibits atypical DNA-binding properties and apurinic-endonuclease activity in vitro. *Nucleic Acids Res.* **35**, 6042-6051.

Lévine, A., Vannier, F., Absalon, C., Kuhn, L., Jackson, P., Scrivener, E., Labas, V., Vinh, J., Courtney, P., Garin, J. and Séror, S.J. (2006). Analysis of the dynamic Bacillus subtilis Ser/Thr/Tyr phosphoproteome implicated in a wide variety of cellular processes. *Proteomics* **6**, 2157-73.

Lopreiato, R., Facchin, S., Sartori, G., Arrigoni, G., Casonato, S., Ruzzene, M., Pinna, L.A. and Carignani, G. (2004). Analysis of the interaction between piD261/Bud32, an evolutionarily conserved protein kinase of Saccharomyces cerevisiae, and the Grx4 glutaredoxin. *Biochem J.* **377**, 395-405.

# Legends to figures

### Figure S1: Spectroscopic analysis of MjKae1/Bud32

**A.** Light absorbance spectra of native MjKae1/Bud32 (a) and PaKae1 (b, Hecker et al. 2007). Spectra were obtained using 200 µg MjKae1/Bud32 or 100 µg PaKae1 in Tris-HCl 20 mM pH 8.0, NaCl 200 mM buffer.

**B.** Electron paramagnetic resonance spectra of MjKae1/Bud32 (**a**) and PaKae1 (**b**, Hecker et al., 2007). The corresponding g-values are indicated for some features. Experimental conditions: microwave power 0.5 mW (top) and 0.1 mW (bottom); microwave frequency 9.38 GHz; modulation amplitude 1 mT; modulation frequency 100 kHz; temperature 10 K. Some g values are indicated on the EPR spectra.

### Figure S2: Sequence alignment of MjKae1 orthologs.

Strictly conserved residues are in white on a black background. Partially conserved amino acids are boxed. Stars below the alignment indicate residues involved in the interface.

### Figure S3: Sequence alignment of MjBud32 orthologs.

Strictly conserved residues are in white on a black background. Partially conserved amino acids are boxed. Filled circles below the alignment indicate residues involved in the interface. The domains of the protein kinases and functionally relevant signatures are indicated below the alignments.

### Figure S4. Superposition of the ATP binding site from MjKae1 and PaKae1.

Stereo view representation of the ATP binding site from MjKae1, colored in blue. Uperposed PaKae1 is colored in yellow with the AMPPNP ligand shown as ball and sticks and the iron ion depicted as a yellow sphere. The 2Fo-Fc electron density map of MJ1130 is shown in blue around the AMPPNP molecule.

### Figure S5. Autophosphorylation of MjBud32 is inhibited by Mj-Kae1.

MJ1130, MjBud32 and/or MjKae1 proteins (approx. 5  $\mu$ g) were subjected to phosphorylation for 30 min. at 72°C in 20  $\mu$ l of a buffer containing 50 mM Hepes-HCl pH 7.5, 500 mM NaCl, 10 mM MnCl<sub>2</sub> and 25  $\mu$ M [ $\gamma$ -<sup>32</sup>P]ATP. Radiolabelled proteins were separated by SDS-PAGE. Gels were then soaked in 16% TCA for 10 min. at 90°C to eliminate radiolabelled nucleotides, stained by Coomassie blue (lower panel) and finally analysed by PhosphorImager (upper panel). **1.** Mj1130 alone, **2.** MjBud32 (**a**) incubated with MjKae1 (**b**), **3.** MjKae1 alone, **4.** MjBud32 alone.

## Figure S6. MjKae1/Bud32 structure.

**A.** Superposition of the two MjKae1/Bud32 protomers present in the crystal asymmetric unit. For clarity, only MjBud32 is shown entirely.

**B.** Ribbon representation of MjKae1/Bud32 monomer with the loops containing insertions in yeast homologues coloured in red (N-terminal part) and green (C-terminal part).

**C.** Molecular surface representation of sequence conservation of Kae1 and Bud32 orthologs. Colouring is from light grey (poorly conserved) to red (highly conserved). AMPPNP is shown as sticks.

Mutation	Primers
Bud32p-R72E	5'-GGCACTAACAAAACAT <u>GAA</u> ACGTTGAATGAGTCGC-3' 5'-GCGACTCATTCAACGT <u>TTC</u> ATGTTTTGTTAGTGCC-3'
Kae1p-D236R	5'-CAGTAAAGGGGATG <u>CGT</u> CTTTCGATGAGTGG-3' 5'-CCACTCATCGAAAG <u>ACG</u> CATCCCCTTTACTG-3'
Kae1p-E292R	5'-CGCCATGCTAGTT <u>CGT</u> ATAACAGAAAGAGC-3' 5'-GCTCTTTCTGTTAT <u>ACG</u> AACTAGCATGGCG-3'
Kae1p-E292,295R	5'-CTATTCGCCATGCTAGTT <u>CGT</u> ATAACA <u>CGT</u> AGAGCTATGGCTCACGTT-3' 5'-AACGTGAGCCATAGCTCT <u>ACG</u> TGTTAT <u>ACG</u> AACTAGCATGGCGAATAG-3'

 Table S1. Primers used for mutant constructs. Mutagenic codons are underlined.

Table S2. Saccharomyces cerevisiae strains used in this study

Strain	Genotype	Source
W303	ura3-1, ade2-1, his3-11,5, trp1-1, leu2-3,112, CAN1-100	
DLY344	as W303, <i>MATa</i> , <i>Akae1::HIS</i> , pFL38-KAE1(URA3)	this study
DLY764	as W303, <i>MATa, BUD32-HA</i> <sub>3</sub> ::HIS	this study
DLY785	as W303, MATalpha, Δbud32::KAN, pFL38-BUD32 (URA3)	this study
DLY1251	as W303, <i>MATalpha</i> , Δ <i>kae1</i> :: <i>HIS</i> , pCM185- <i>KAE1(TRP1)</i>	this study
DLY1252	as W303, <i>MATalpha</i> , Δ <i>kae1</i> :: <i>HIS</i> , pCM185- <i>kae1D236R (TRP1)</i>	this study
DLY1253	as W303, <i>MATalpha</i> , Δ <i>kae1</i> :: <i>HIS</i> , pCM185- <i>kae1E292R (TRP1)</i>	this study
DLY1254	as W303, <i>MATalpha</i> , Δ <i>kae1</i> :: <i>HIS</i> , pCM185- <i>kae1E295R (TRP1)</i>	this study
DLY1255	as W303, MATalpha, Δkae1::HIS, pCM185-kae1EE292-295RR (TRP1)	this study
DLY1261	as W303, MATalpha, Δbud32::KAN, pCM185-BUD32 (TRP1)	this study
DLY1264	as W303, MATalpha, Δbud32::KAN, pCM185-bud32K57R60EE (TRP1)	this study
DLY1262	as W303, MATalpha, Δbud32::KAN, pCM185-bud32R72E (TRP1)	this study
DLY1263	as W303, MATalpha, Δbud32::KAN, pCM185-bud32R204E (TRP1)	this study

Figure S1



Β.



Minnaachii		β1	β2	_	β3	α1
Mjannaschil	1	10	20		30 40	50
Mjannaschii Mmaripaludis	MDNSKDDI	ICIGFEGTAE	KSGVGIIITS	KGE <b>VL</b> FI	NKTINYKPPKQGINP NKTIIYTPPVQGIHP	REAADHHAETFPK RE <b>AA</b> DHHAETFVK
Mthermoautotrophicum Pabyssi		LCLGIEGTAE LALGIEGTAH	TLGIGIVSE		L <b>R</b> GKP <b>LI</b> PEKG <b>GI</b> H <b>P</b> N <b>V</b> FDT <b>LT</b> TEKG <b>GI</b> H <b>P</b>	REAAEHHAKWIPR KE <b>AA</b> E <mark>HH</mark> ARLMKP
Phorikoshii Ecuniculi		ILALGIEGTAH IAMGLEGSAN	TLGIGIVSE KLGVGIMRD		N <b>V</b> FDT <b>LT</b> TEKG <b>GI</b> HP E <b>R</b> LTY <b>AP</b> PPGE <b>GF</b> I <b>P</b>	KE <b>AA</b> E <b>HHAR</b> LMKP VK <b>TA</b> E <b>HHRS</b> RILG
Hsapiens Xlaevis	MP	AVLGFEGSAN IVVGFEGSAN	KIGVGVVRD		PRRTYVTPPGTGFLP PRRTYITPPGOGFMP	GD <b>TA</b> R <b>HHRA</b> VILD SD <b>TA</b> R <b>HHRS</b> CILD
Mmusculus	ME	AVLGFEGSAN	KIGVGVVRD	GTVLAN	PRRTYVTAPGTGFLP	GDTARHHRAVILD
Ylipolytica	MTTY	LSLGLEGSAN	KLGVGVIKHTVT	DANAENGFSTDI <b>LS</b> N	IRDTYITPPGEGFLP	RDTARHHRNWVVR
Agossypii Scerevisiae	MVNLNKVQPKSGGDHY	IALGLEGSAN	KLGVGILKHPML KLGVGIVKHPLL	PKHANSDLSYDCEAEM <b>LS</b> N	IRDIYITPPGEGELP IRDIY <b>VT</b> PPGE <b>GE</b> LP	RDTARHHRNWVVR RD <b>TA</b> RHHRNWCIR
	1 10	20	30	40 50	60 70	80
		β4	α2	β5	α3	β1'
Mjannaschii	eò	7 <u>0</u>	s o	90 100	110 120	130
Mjannaschii Mmaripaludis	LIKEAFEVVDK N	EIDLIAFSOG	PGLGPSLRVTAT	VARTLSLTLKKPIIGVNHC	IAHIBIGKLTTEAED	PLTLYVSGGNTQV PLTLYVSGGNTQV
Mthermoautotrophicum	LIAEACRDAGVELG	EIGLISFSRG	PGLGPALRTVAT.	AARTLALSLDVPIVGVNHC	IGHIDIGRLTTGASD	PVSLYVSGGNTOV
Phorikoshii	LLKKALEKAGISMD	DIDVIAFSQG	PGLGPALRVVAT	AARALAIRYNKPIVGVNHC	IAHVEITKMFG.IKD	PVGLYVSGGNTQV
Hsapiens	LLQEALTESGLISQ	DIDCIAYTKG	PGMGLPLSVVAT PGMGAPLVSVAV	VARTUAQLWNKPLVGVNHC	IGHIEMGRLITGATS	PTVLYVSGGNTQV
Xlaevis Mmusculus	VLQEALEESNIKPE LLQEALAEAGLTSK	DVDCVAYTKG DIDCIAFTKG	PGMGAPLLSVAI PGMGAPLASVAV	VARTVAQLWKKPLLGVNHC VARTVAQLWNKPLLGVNHC	IGHIEMGRLITGAEN Ighiemgrlitgavn	PTVLYVSGGNTQV PTVLYVSGGNTQV
Spombe Ylipolytica	LIKQAFAEAKISFK IIKRALDEAKISDP.T	DIDCICFTKG KLHCISFTQG	PGIGAPLNSVAL PGMGAPLOSVVI	CARMLSLIHKKPLVAVNHC AARTIAOMWGVPLVGVNHC	IGHIEMGREITGAQN VGHIEMGRTITGATN	PVVLYVSGGNTQV PVVLYVSGGNTQV
Agossypii Scerevisiae	LVRRALVEAGIEDP.F	LDVICFTKG	PGMGAPLHSVVV. PGMGAPLHSVVI	AARTMSMLWDVPLVAVNHC AARTCSLLWDVPLVGVNHC	IGHIEMGREITKAEN Ighiemgreitkaon	PVVLYVSGGNTQV PVVLYVSGGNTOV
	90 100	110	120	130 140	150	160 170
	β2' β3'	αΙ		αll		αШ
Mjannaschii	140 150	00000000	222 ع 170 ع	180	<u>0000</u> 190 200	210
Mjannaschii	IAYVSKKYRVFGETL	IAVGNCLDOF	ARYVNIPHPGGP	. Y <b>iee</b> l <b>ark</b> gkkl <b>v</b> d	LPYTVKGMDIAFSGL	TAAMRAYDAG .
Mmaripaludis Mthermoautotrophicum	IAFNEGRYRVIGETLI IAFNEGRYRVFGETLI	IAIGNCLDOF	ARHCNLPHPGGV ARESGLGHPGGP	VIEQLALKASEYIE	LPYTVKGMDLSLSGL LPYSVKGMDISFSGL	TAALRKMEAG
Pabyssi Phorikoshii	LALEGGRYRVFGETLD LALEGGRYRVFGETLD	IGIGNAIDVF IGIGNAIDVF	ARELGLGFPGGP ARELGLGFPGGP	KVEKLAEKGEKYIE K <b>lek</b> l <b>aek</b> gknyid	LPYAVKGMDLSFSGL LPY <mark>AVKGMDLSF</mark> SGL	TEAIRKYRSGK. TEAIRKYRSGK.
Ecuniculi Hsapiens	IAYHNRRYKIFGETLI IAYSEHRYRIFGETI	IAVGNCIDRF	ARALKLPNFPAP ARVLKISNDPSP	GLS <b>VER</b> Y <b>AKL</b> GKNYIE GYN <b>IEQ</b> M <b>AKR</b> GKKL <b>V</b> E	LPYVVKGMDVSFSGI LPYTVKGMDVSFSGI	ISNIKRKIAEDEQ ISFIEDVAHRMLA
Xlaevis Mmusculus	IAYSERCYRIFGETID ISYSEHRYRIFGETID	IAVGNCLDRF	ARVLKISNDPSP ARVLKISNDPSP	GYN <b>IEOMAKK</b> GKKF <b>V</b> E GYN <b>IEOMAKR</b> GKKL <b>V</b> E	LPYTVKGMDVSFSGI LPYTVKGMDVSFSGI	SYIEDMSHKMLS SFIEDAAORMLA
Spombe Vlipolytica	IAYSEKKYRIFGETLU	IAIGNCLORF	ARIIGLSNAPSP ARVIKTPNAPSP	GYNINCEAKKGKRFIE	LPYTVKGMDCSFSGL	SGVEAAATELLD
Agossypii	IAYSENRYRIFGETL	TAIGNCLORF	ARTLKIPNDPSP	GYNIEOLAKOCKNKDRLVE	LPYTVKGMDLSMSGI	AHIDSLAKDLFR
SCELEVISIAE	180	190	200 2	10 220	230 240	250
	*		a1'	★ ★ β4'	* ** *	85'
Mjannaschii		2000000	20000000000	240 250	200000000000	$\chi \rightarrow 280$
Mjannaschii		. ERLEDICYS	LQEYAFSMLTEI	TERALAHTNKGEVMLVGGV	AANNRIREMIKAMCE	GQ.NVDFY <b>V</b> PPK <b>E</b>
Mmaripaludis Mthermoautotrophicum		. ERIEDVCYS . ASLEDLAYS	LOETSFSMLTEI IOETAFSMLVEV	TERALAHTNKAEVMLVGGV TERALAYTEKNQVLLCGGV	A ANNRIKEMIKVMCE A VNRRIRDMIREMCO	EQ.NVDFY <b>V</b> PEK <b>Q</b> EH.HVEFH <b>M</b> PPP <b>E</b>
Pabyssi Phorikoshii		. YRVEDLAYS	FOETAFAALVEV FOETAFAALVEV	TERAVAHTEKDEVVLVGGV TERALAHTEKKEVVLVGGV	AANNRLREMLRIMTE AANNRLREMLKIMAE	DR.GIKFF <b>V</b> PPY <b>D</b>
Ecuniculi		VKRDLCYS	LOETVESALVEV	TERAMAFSSSKEVLIVGGV	GCNLRLOEMMGIMAR	ER.GGVCYATDER
Xlaevis		ECTPEDICFS	LOETLESMLVEI	TERAMAHCGSQEVLIVGGV	GCNVRLOEMMGVMCE	ER.GAKIFATDER
Spombe	P	SVTKODLCYS	LOETGEAMLVEI	TERAMAHIRADSVLIVGGV	GCNERLQQMMAEMSS	DR.GADVFSTDER
Agossypii	RNTKNYKLFDRETGKO	LVTVEDLCYS	LOBILFAMLVEI	TERAMAHVNSQQVLIVGGV TERA <mark>M</mark> AHVNSNQVLIVGGV	GCNERLQEMMGIMAR GCNVRLQQMMASMCQ	SRADGQVH <b>A</b> TDE <b>R</b>
Scerevisiae	GNKKNKILFDKTTGEG	KVTVEDLCYS 280	LOENLEAMLVEI 290	300 310	GCNVRUQEMMAQMCK 320	DRANGQVHATDNR 330 340
	-21	*	** * **	*	* * **	** *
Mjannaschii	00000000000000000000000000000000000000	00	21.0			
Mjannaschii	290 FCGDNGAMIAWLGLLM	HKNG.RWMSL	JIO DETKIIPNYRTA	320 330 MVEVNMIKEIKGKKRKI		
Mmaripaludis Mthermoautotrophicum	FCGDNGAMIAWLGILC	YLNG.KRMDL	KDTKPISNYRSD	MVEVNWIHDESKNLNDGNI	KSRKI	
Pabyssi	LCRDNGAMIAYTGLR	YKAG.ISFRL	EETIVKOKFRID			
Ecuniculi	FCIDNGVMIAYVGML	AKSG.AAFKL	GECFVTQRYRTD	SVEVTWRDY		
Hsapiens Xlaevis	FCIDNGAMIAOAGWEM FCIDNGAMIAOAGWEM	IF <b>R</b> A <b>G.</b> HRTP <b>L</b> IF <b>R</b> A <b>G.</b> QVTN <b>L</b>	SDSGVTQRYRTD QDSWITQRYRTD	EVEVIWRD	MAAARATT	
Mmusculus Spombe	FCVDNGAMIAQAGWEM FCIDNGIMIAQAGLLA	IFQAG.HRTPL	KDSAITQRYRTD AESTITORYRTD	EVEVTWRD		
Ylipolytica Agossypii	FCIDNGIMIAHAGLLA	WROG.FETKM	EKTQCTORFRTD			
Scerevisiae	FCIDNGVMIAOAGLIE	YRMGGIVKDE	SETVVTOKFRTD	e <b>v</b> y <b>a</b> a₩rd		
	350	360	370 3	80		









C.

