Supplemental Table 1. MPP-homologous genes in archaeal and eubacterial genomes

Domain	Genomes	βMPP-like genes*	αMPP-like genes*
Archaea	29	1	1
Eubacteria	511	487	117 (No GRLs)
Rickettsia	20	33	19 (No GRLs)

*The expect values under $1E^{-10}$ are counted after BLAST search, using yeast βMPP as a query

Legends for Supplemental Figures

Supplemental Fig. 1. Expression and purification of RPP. *A*, RT-PCR analysis for the RPP gene. The gene-specific first primers and PCR primer pairs are shown as long and short arrows, respectively. The RT-PCR products in the presence or absence of reverse transcriptase (RT: + or -) were separated by agarose gel electrophoresis and stained with ethidium bromide. The PCR product for GroEL was electrophoresed following 10-fold dilution compared to that for RPP. *B*, Immunoblotting analysis of RPPs. Following independent recovery of Vero cells infected by different *Rickettsia* species, the proteins were separated by SDS-PAGE and detected by western blotting using an anti-RPP polyclonal antibody. Rec., His_6-tagged recombinant protein. *C*, Purification of recombinant RPP. RPP was expressed in *E. coli* BL21 and purified as described in the Materials and Methods. The proteins were separated by SDS-PAGE and either stained with Coomassie Brilliant Blue R-250 (*upper*) or detected by western blotting using an anti-RPP polyclonal antibody (*lower*). C, control non-transformed *E. coli* BL21 cells; T, total cells transformed with pET-RPP; P, pellets obtained following centrifugation at 10,000 x *g* after cell lysis; S, supernatants obtained following centrifugation at 10,000 x *g* after cell lysis. Lanes 1, 2 and 3 show the eluates from the nickel-chelating, first DEAE-Sepharose and second DEAE-Sepharose columns, respectively.

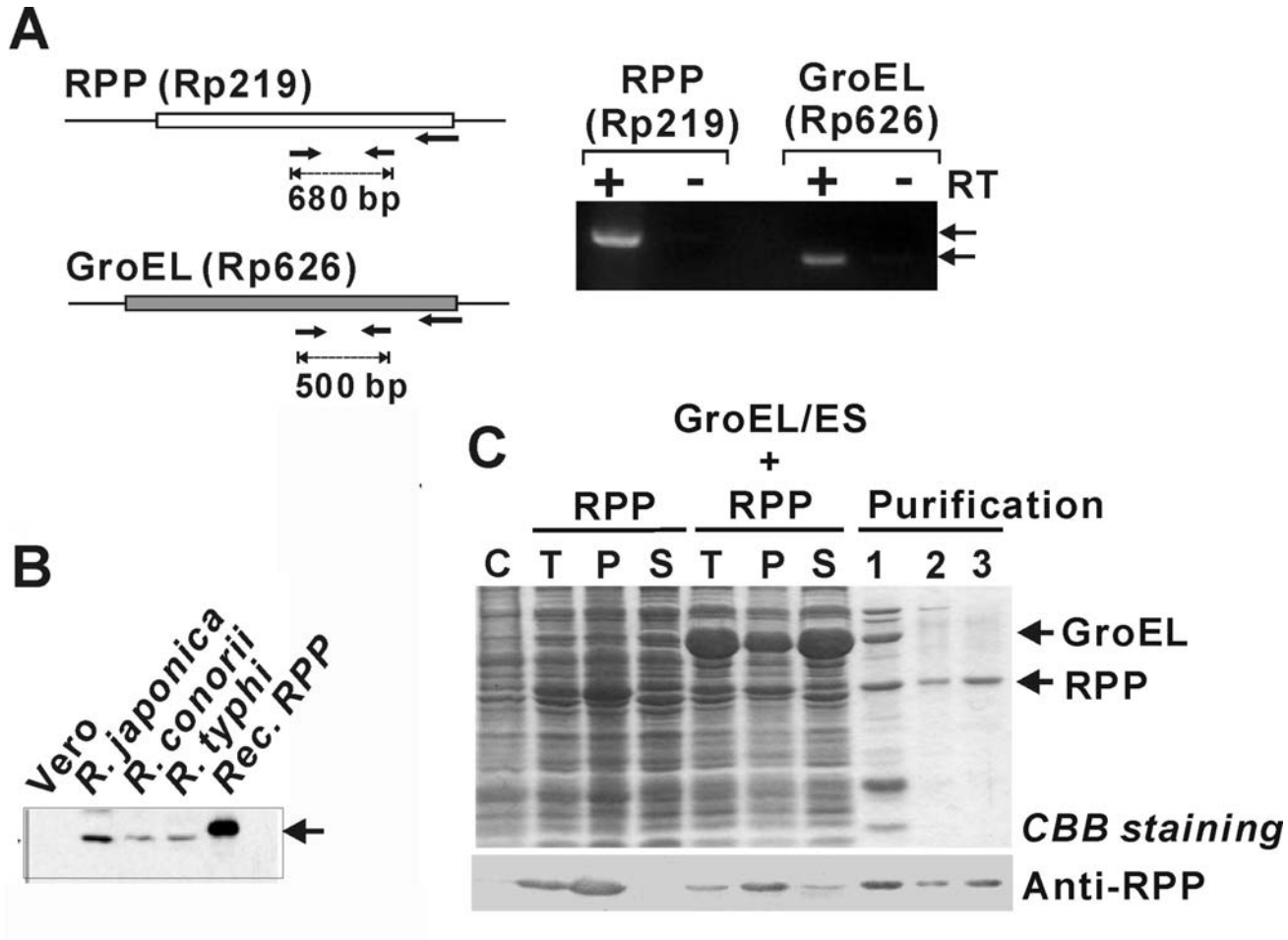
Supplemental Fig 2. Reverse-phase HPLC analyses of the peptide cleavage activity of RPP. Peptides were incubated in the presence (+) or absence (-) of RPP and separated as described in the Materials and Methods. Arrows indicate the peptide fragments cleaved by RPP. VIP: vasoactive intestinal protein.

Supplemental Fig 3. Action of RPP toward basic proteins. Native or denatured proteins (12.5 μg/ml) were incubated with (+) or without (-) RPP (1.25 μg/ml) in the processing buffer at 30ºC for 60 min, separated by SDS-PAGE and silver-stained. The protein denaturation was performed in 10 mM HEPES-KOH (pH 7.5) containing 8 M urea and 10 mM DTT before the incubation with RPP, and the denatured proteins were rapidly diluted by 100-fold in the processing buffer. Sub: substrate proteins.

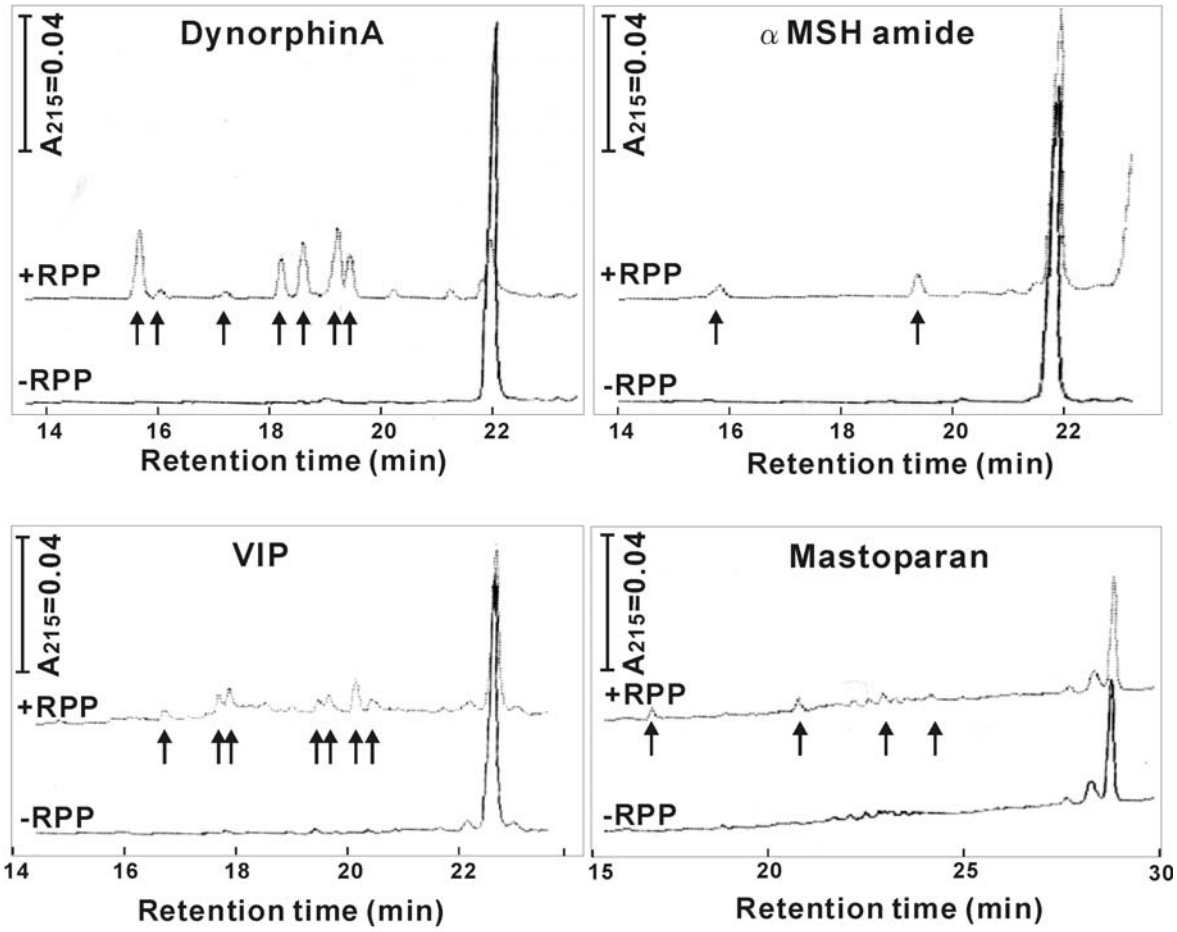
Supplemental Fig 4. Reverse-phase HPLC analyses of presequence peptide cleavage by RPP. Synthetic peptides were incubated in the presence (+) or absence (-) of RPP or MPP and separated by reverse-phase HPLC as described in the Materials and Methods. Arrows indicate the peptide fragments cleaved by RPP and MPP. Enz: enzyme.

Supplemental Fig 5. Reverse-phase HPLC analyses of preMDH peptide cleavage by RPP and its mutant. Synthetic peptides of MDH_{2-28} were incubated with RPP, RPP* (E52Q mutant) and MPP, and then separated by reverse-phase HPLC as described in the Materials and Methods. Cleaved peptides were identified by MALDI-TOF mass spectrometry and their sequences are shown at the bottom.

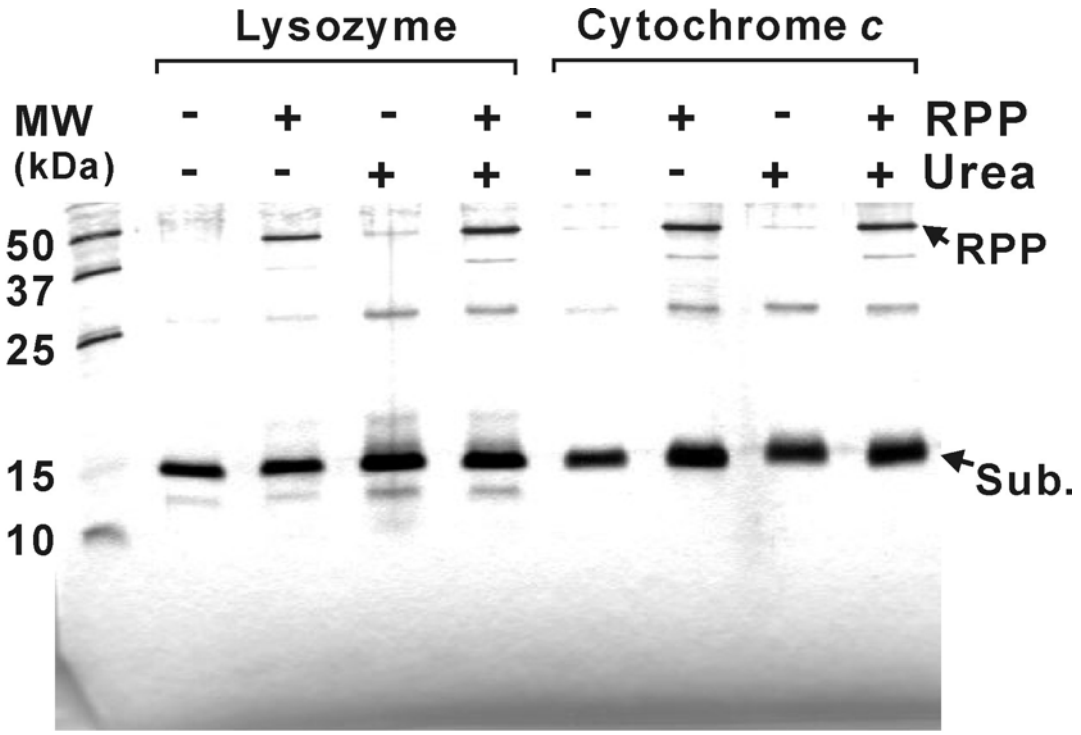
Supplemental Fig 6. Evolution of MPP from a progenitor peptidase in an ancient parasite. See the Discussion for details. The M16 peptidase, porin and permease encoded in the parasitic genome are converted into distinct component proteins of a transport-processing system, which assemble and work systematically with each other.



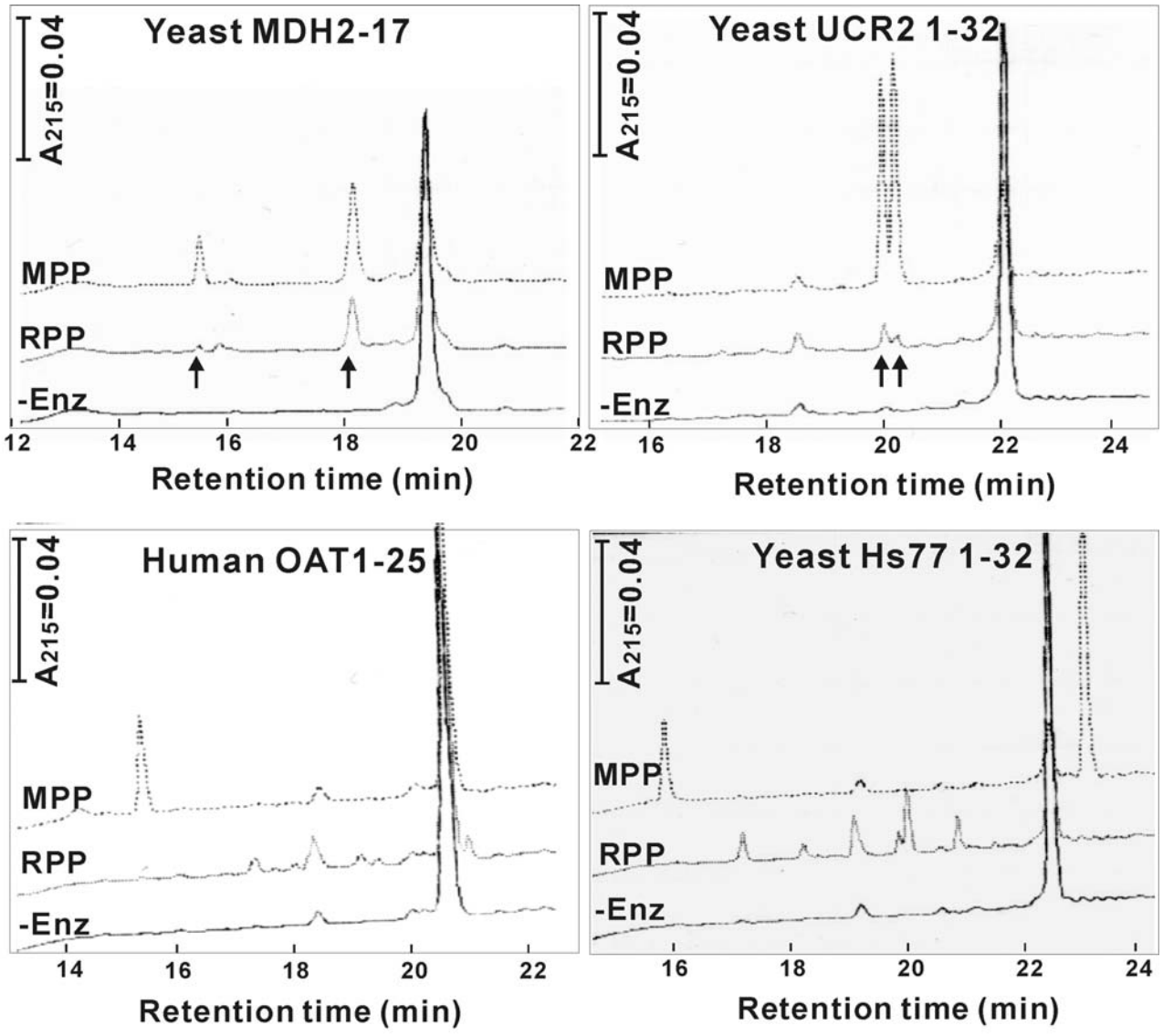
Supplemental Figure 1



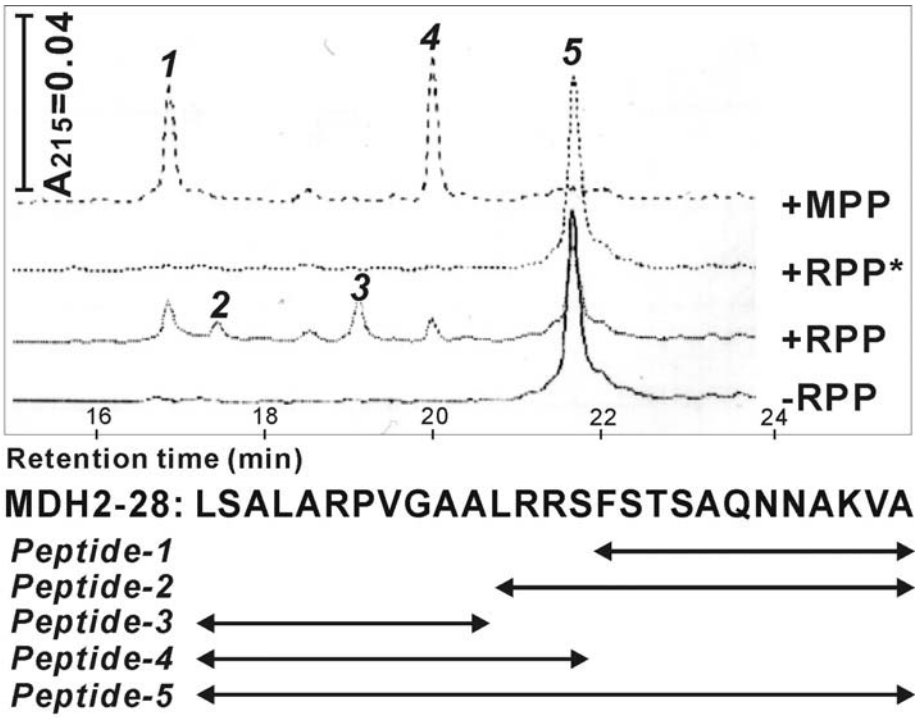
Supplemental Figure 2



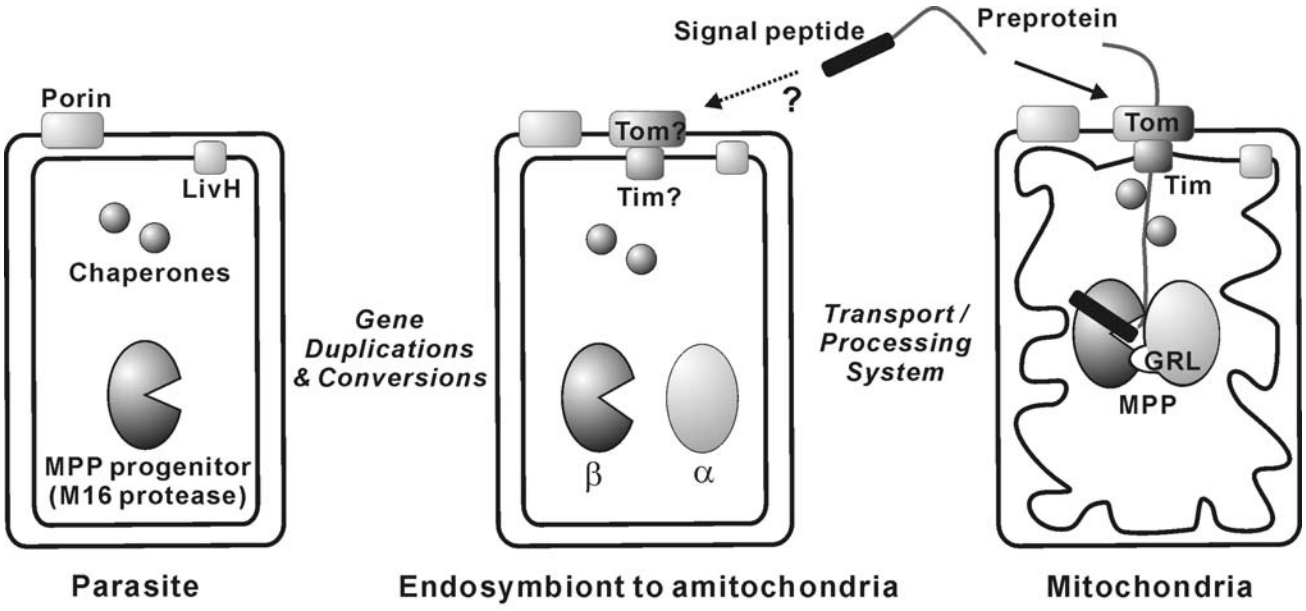
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6