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BJ Metabolism

Biochem. J. (2012) **443**, 535–547 (Printed in Great Britain) doi:10.1042/BJ20111478

online data

SUPPLEMENTARY ONLINE DATA

Novel structural arrangement of nematode cystathionine β-synthases: characterization of *Caenorhabditis elegans* CBS-1

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Figure S1 Unrooted tree of fold-type II PLP-dependent proteins with ten CBS homologues in *C. elegans*

For phylogenetic analysis, we used various proteins from the family of fold-type II PLP-dependent proteins: CBS_Human (UniProt entry P35520), CBS_Rat (UniProt entry P32232), CBS_Drosophila (UniProt entry Q9VRD9), CBS_Trypanosoma (UniProt entry Q9BH24), CBS_Saccharomyces (UniProt entry P32582), CYSK_Saccharomyces (UniProt entry P53206) CYSK_Arabidopsis (UniProt entry P47998), CYSM_E. coli (UniProt entry P16703), CYSK_E. coli (UniProt entry P0ABK5), SDSL_Human (UniProt entry Q96GA7), THDH_Saccharomyces (UniProt entry P00927), THD1_E. coli (UniProt entry P04968) and THD1_Arabidopsis (UniProt entry Q9ZSS6). Ten CBS homologues (bold font) are presented in Table S1. The numbers at the internal nodes represent bootstrapped values (maximum 100). The upper left-hand edge in red denotes the CBS branch. The tree topology demonstrates three separated groups for ten CBS homologues in *C. elegans*: ZC373.1 and F54A3.4 belong to the CBS branch, C17G1.7, R08E5.2, F59A7.9 and K10H10.2 belong to the cysteine synthase A branch, and the remaining homologues belong to other fold-type II PLP-dependent protein families. CYSK, cysteine synthase A; CYSM, cysteine synthase B; SDSL/THD, serine/threonine dehydratase family.

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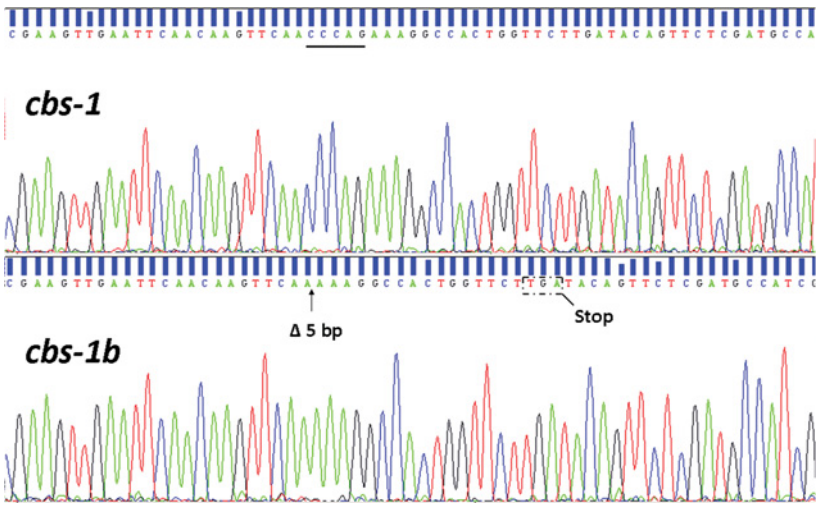

Figure S2 Sequences of *cbs*-1 RT–PCR products covering the exon 6 and 7 junction

Two splice variants of *cbs-1* were found. The novel *cbs-1b* transcript leads to a frameshift and a subsequent stop codon that allows for translation of the separated N-terminal module of CBS-1.

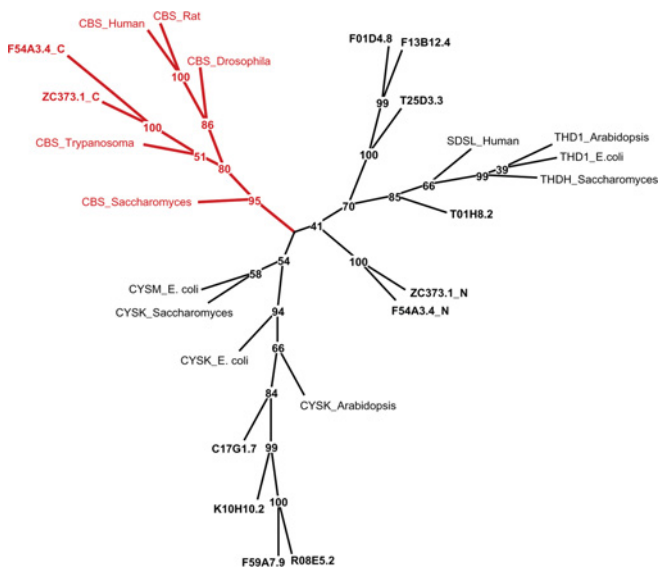

Figure S3 Unrooted tree of separated conserved regions from CBS-1 with various fold-type II PLP-dependent proteins

This tree is taken from the same phylogenetic study as that presented in Figure S1, with the exception that the two conserved regions from CBS orthologues ZC373.1 and F54A3.4, were separated for the analysis. The topology of the unrooted tree demonstrates that the N-terminal regions of CBS homologues (ZC373.1_N and F54A3.4_N) do not belong to any branch containing the analysed proteins, whereas the C-terminal regions (ZC373.1_C and F54A3.4_C) belong to the CBS branch. Alignment of the N-terminal module of *C. elegans* CBS-1 (the first of the two tandemly arranged regions, i.e. residues 14–322) revealed 29 % identity and an e-value of 5e − 27 compared with the catalytic core of human CBS (residues 72–397), whereas the C-terminal module (i.e. residues 374–702 of CBS-1) revealed much higher 54 % identity and an e-value of 2e − 99.

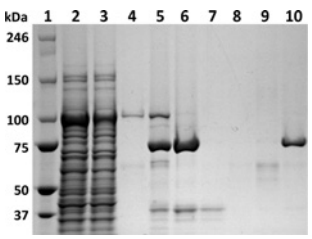

Figure S4 CBS-1 purification procedure

The purification procedure for CBS-1 is illustrated by a 3–8 % SDS-containing polyacrylamide gel stained with Coomassie EZ Blue. Lane 1, molecular mass markers; lane 2, bacterial extract after centrifugation; lane 3, flow-through fraction from glutathione–Sepharose column; 4, wash fraction of glutathione–Sepharose column; lane 5, fusion protein that was cleaved by PreScission protease; lane 6, elution of CBS-1 after on-column cleavage; lane 7, flow-through fraction from the Ni-Sepharose column; lanes 8 and 9, wash fractions of the Ni-Sepharose column by IMAC buffer containing 20 mM and 50 mM imidazole respectively; lane 10, elution of CBS-1 by IMAC buffer containing 75 mM imidazole. The molecular mass is given in kDa on the left-hand side.

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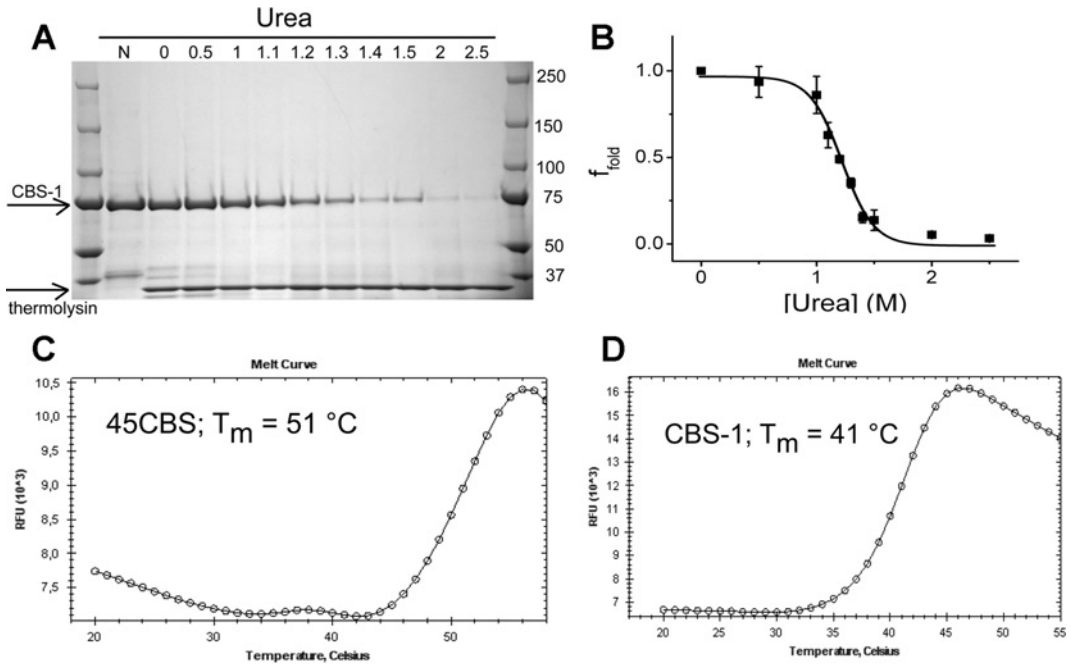

Figure S5 Pulse proteolysis and fluorescence-based thermal-shift assay

Pulse proteolysis in a urea gradient employing thermolysin and thermal-based assays were used to determine possible differences in enzyme stability between CBS-1 and human 45CBS. (**A**) Representative SDS/PAGE gel. The molar concentration of urea for the proteolytic pulse is indicated at the top of each lane and the molecular mass is given in kDa on the right-hand side. (**B**) F-fold values, which represent the fraction of the remaining intact protein after the proteolytic pulse, are plotted against the urea concentration. Results are means ± S.D. from three measurements and the curves were fitted by non-linear regression. (**C** and **D**) Melting curves in fluorescence-based thermal shift assays reveal melting points (T_m) of 51 °C and 41 °C for human 45CBS and CBS-1 respectively.

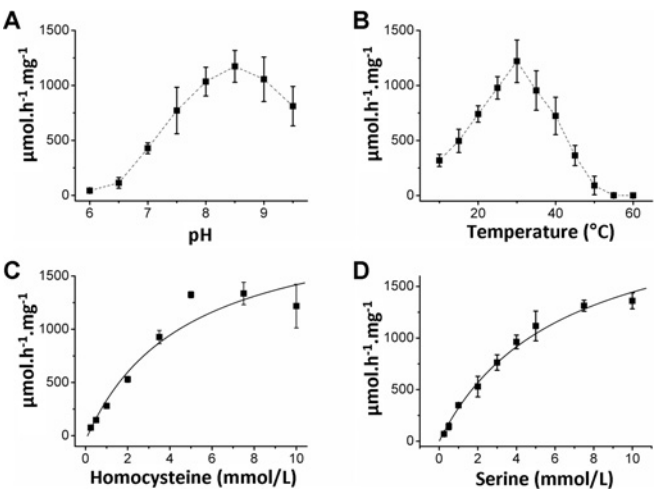

Figure S6 Enzymatic properties of recombinant CBS-1

(**A** and **B**) The dependence of CBS activity on pH and temperature respectively. The kinetic properties of CBS-1 for 1–10 mM homocysteine in a mixture with 10 mM serine are shown in (**C**), whereas the properties for 1–10 mM serine in a mixture with 10 mM homocysteine are shown in (**D**). Results are means ± S.D. from four measurements.

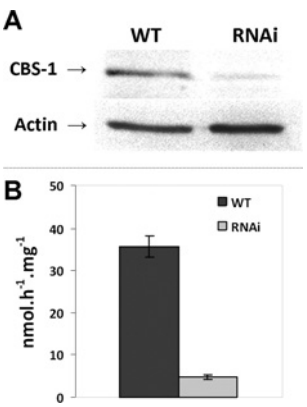

Figure S7 Western blot analysis and CBS assay of crude nematode extracts

(**A**) Western blot analysis showing a decreased level of CBS-1 protein in nematodes after RNAi. Actin was used as a reference protein. (**B**) CBS activity is significantly decreased in nematodes after RNAi. Results are means ± S.D. from two independent measurements.

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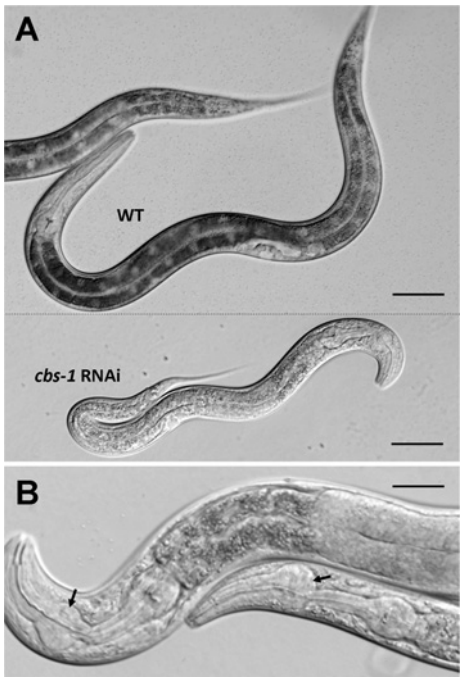

Figure S8 Inhibition of *cbs-1* by RNAi

Images show the body morphologies of worms in Nomarski optics. (**A**) L4 stage of *cbs-1* RNAi and WT worms. The affected worms exhibit decreased body mass and partial lack of pigment granules in the intestine. Scale bars, 50 μm. (**B**) Higher magnification of an affected adult nematode pharynx. The pharynx shows abnormal morphogenesis of the metacorpus with a balloon-like appearance indicated by an arrow. Scale bar, 25 μm.

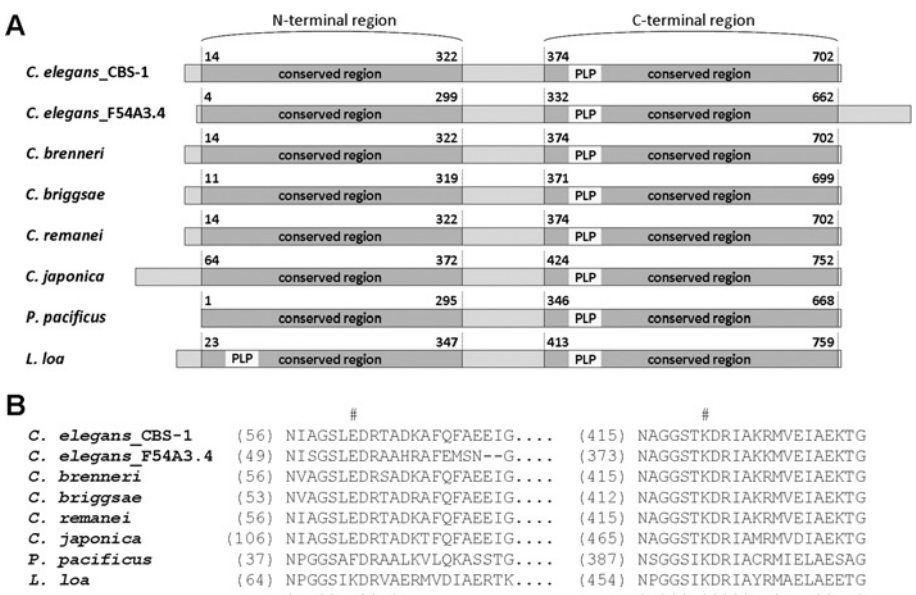

Figure S9 Domain architecture of CBS enzymes in nematodes

(**A**) Domain organization of nematode CBSs. The predicted amino acid sequences of hypothetical nematode CBSs were aligned with the sequence of *C. elegans* CBS-1, and the domain architecture of the proteins was inferred from the degree of homology. Primary structures are aligned by the PLP-binding lysine residue. The numbers indicate the first and the last amino acid residues in each of the conserved domains. Hypothetical proteins included in the alignment are as follows: *C. elegans*_ZC373.1 (CBS-1, UniProt entry Q23264); *C. brenneri* (WormBase accession number CN28558); *C. briggsae* (UniProt entry A8WRM3); *C. remanei* (UniProt entry E3MMP8); *C. japonica* (WormBase accession number JA15528); *C. elegans* F54A3.4 (UniProt entry Q9N4K2); *Pristionchus pacificus* (WormBase accession number PP12619); *Loa loa* (UniProt entry E1FTU4). (**B**) Amino acid alignment of hypothetical PLP-binding site of separated N-terminal and C-terminal conserved regions of various nematode CBSs. #, site of the putative PLP-binding lysine residues. $*$, conserved residue. Only *Loa loa* CBS contains a lysine residue in both PLP-binding sites.

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Table S1 Primer sequences

Primers A–F were used to generate the cbs-1–GFP vector (see the GFP reporter assay section of the main text), G and H were used for the amplification of the shortened *cbs-1* coding sequence (see the RNA-mediated interference section of the main text), I–K (sense) and L–O (antisense) were used in combination to amplify the *F54A3.4* open reading frame (see the PCR amplification and sequencing section of the main text), P–U (sense) and V (antisense) were used for the amplification of the *cbs-1* coding sequence with specific cloning overhangs (see the Bacterial expression and purification section of the main text). Primers W–Z were used for site-directed mutagenesis.

Primer	Sequence
A, cbs-1_S	5′-ACTTGACGGAAAAGCTGGCAGA-3′
B, cbs-1_A	5′-AGTCGACCTGCAGGCATGCAAGCTGGCGTCTAGGAAATGACGCTCATTC-3′
C, GFP_S	5′-AGCTTGCATGCCTGCAGGTCG-3′
D, GFP_AS	5′-AAGGGCCCGTACGGCCGACTA-3′
E, cbs-1_S*	5′-GAGGAATGACCATCAATTTGA-3′
F, GFP_AS*	5′-GGAAACAGTTATGTTTGGTATA-3′
G , RNAi_S	5′-GACCCTCATGGATCTATTC-3′
H, RNAi_AS	5′-GACGCTCATTCATCCAATC-3′
I, F54_S1	5′-GACGAATTCATGTGCCTGCCTACCATTAAA-3′
J, F54_S2	5′-GGCAAGACGCCACTGGTGAA-3′
K, F54_S3	5′-AGAAGACAACAGTGGTCGGAGTGAGAT-3′
L, F54_AS1	5′-CAAGCGGCCGCTCAATAGAAAATGCGAGAGCG-3′
M, F54_AS2	5′-AGAGATTCCGGTGATGGTAC-3′
N, F54_AS3	5′-CAACGGCACCAGTTGAGTTG-3′
O, F54_AS4	5′-TGGCTTCCAGCACTGCCGC-3′
P, CBS-1_S	5′-CCTGGGATCCATGATCCAAAACGAAGTTTCC-3′
Q, Δ1–372	5′-CCTGGGATCCCCAGAAAGGCCACTGGTTCTT-3′
R, Δ1–322	5′-CCTGGGATCCGTGGTGACCAGAAAAGATGGA-3′
S, Δ1–299	5′-CCTGGGATCCATGGAATTAGAAATTATC-3′
T, b/375	5′-CCTGGGATCCATGGACCACAACCAAACAGCA-3′
U, b/360	5′-CCTGGGATCCATGATCCAACTAAACTTGCTG-3′
V, CBS-1_AS	5′-GCCGCTCGAGTTAGTGGTGATGGTGATGATGGGCGTCTAGGAAATGACG-3′
W, K421A_S	5′-TGAACGCTGGGGGATCAACAGCGGATCGTATTG-3′
X, K421A_AS	5′-CATTCTTTTGGCAATACGATCCGCTGTTGATCCC-3′
Y, E62K_S	5′-TCAATATTGCGGGATCTTTGAAAGACCGTACCG-3′
Z, E62K_AS	5′-GCTTTGTCAGCGGTACGGTCTTTCAAAGATCCC-3′

Table S2 Homologous level of CBS-related proteins in *C. elegans*

C. elegans genes are arranged by the level of homology with human CBS. The query sequences are as follows: human CBS (UniProt entry P35520), trypanosomal CBS (UniProt entry Q9BH24) and bacterial CS (UniProt entry P0ABK5). In each group of comparisons, the left-hand column lists the e-value, and the middle and right-hand column list identical and positive matches in percentages respectively. The Table shows three groups of ten CBS homologues in *C. elegans*: ZC373.1 and F54A3.4 have the highest homology with CBS; C17G1.7, R08E5.2, F59A7.9 and K10H10.2 are the most homologous with cysteine synthase; and the remaining homologues belong to other unspecified fold-type II PLP-dependent protein families. AA, number of amino acids in the hypothetical protein; COG, clusters of orthologous groups of proteins; CBS RE, CBS and related enzymes; NA, not assigned; SR, serine racemase.

			Homology [e-value, identities (%), positives (%)]								
Name	AA	COG	human CBS (551 AA)			*T. cruzi* CBS (384 AA)			*E. coli* CS (323 AA)		
ZC373.1	704	CBS RE	8e − 94	54	71	2e − 84	50	66	2e − 30	30	44
F54A3.4	755	CBS RE	4e − 92	54	68	8e − 87	51	66	6e − 27	32	46
C17G1.7	341	NA	2e − 54	44	60	4e − 41	36	51	5e − 62	44	59
K10H10.2	337	CBS RE	4e − 54	38	60	9e − 43	35	56	1e − 60	45	58
R08E5.2	337	CBS RE	1e − 51	37	58	2e − 42	36	55	1e − 57	41	57
F59A7.9	337	CBS RE	7e − 42	36	58	3e − 38	35	54	4e − 56	41	55
F01D4.8	430	CBS RE	2e − 10	24	43	0.012	25	49	0.043	26	46
T25D3.3	427	CBS RE	2e − 09	25	40	0.003	24	44	0.001	28	56
T01H8.2	317	SR	1e − 07	28	43	2e − 04	23	39	0.015	29	48
F13B12.4	435	CBS mRE	5e − 07	21	40	0.037 23	47	5e-04	26	45	

Received 15 August 2011/13 December 2011; accepted 13 January 2012
Published as BJ Immediate Publication 13 January 2012, doi:10.1042/BJ20111478

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