

Recoding UAG to selenocysteine in *Saccharomyces cerevisiae*

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AsSclA:

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***tS(AGA)D2* (including -500bp and +300bp of the tRNA coding sequence):**

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***ADH1-sfGFPS2Am-RPS20*:**

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hMsrB1 (95TAG)

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SUPPLEMENTARY FIGURES

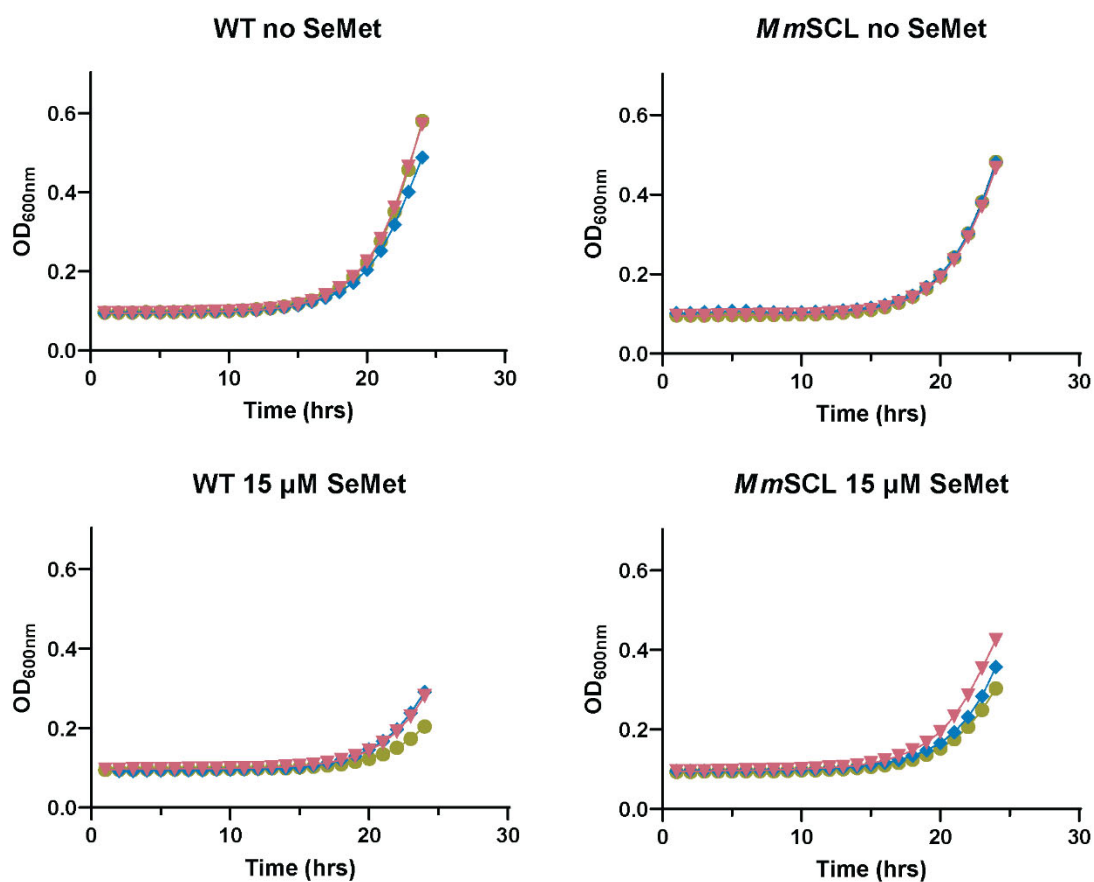


Figure S1. Expression of *Mus musculus* selenocysteine lyase (*MmSCL*) reduces toxicity of SeMet in yeast. Three replicates of cell growth shown over the course of 24 hours in conditions without (WT) and with *MmSCL*.

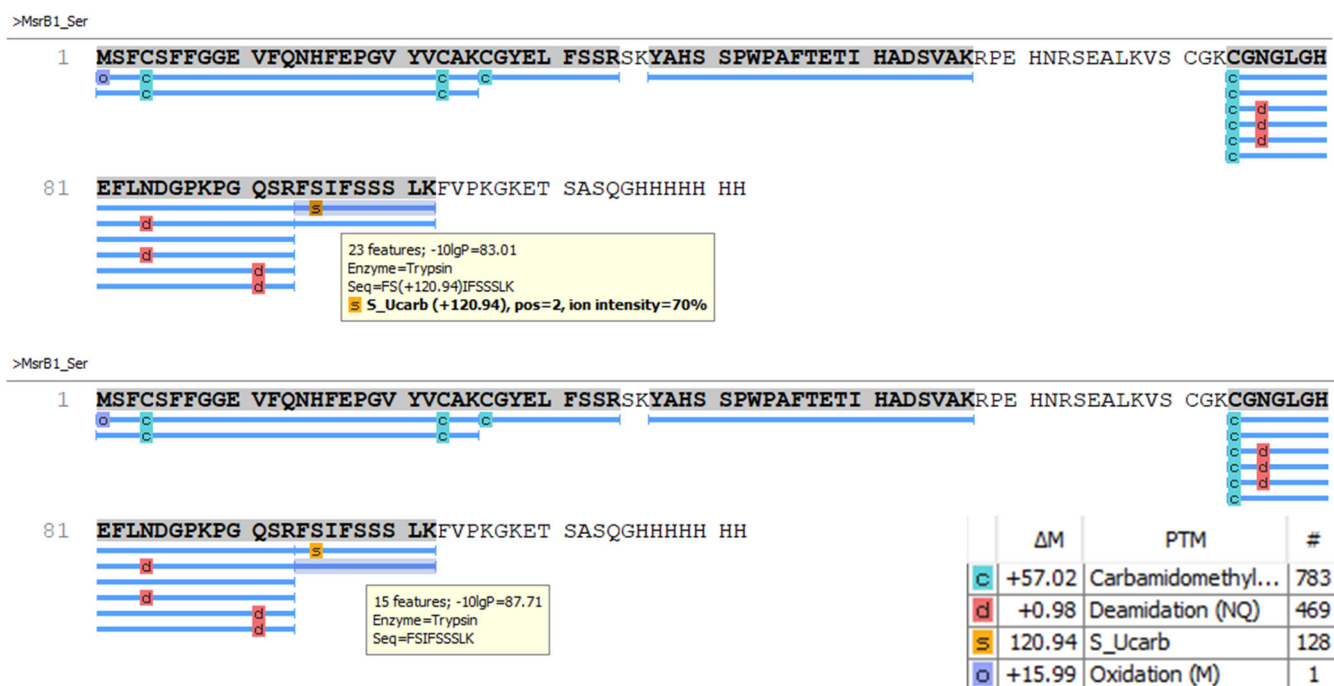


Figure S2. Peptide mapping to MsrB1. Following tryptic digest of MsrB1 and tandem MS, peptide data shows insertion of Sec (orange s) at the correct position.

TABLES

Table S1. Quantification of MsrB1 selenium occupancy by ICP-MS^a

MsrB1 ^b sample replicate	82-Se [NoGas] Final Conc (µg/L)	Total Volume (L)	Mass of Protein (µg)	82-Selenium Conc. (µg/g protein)	Sec occupancy in MsrB1
0 ^c				5416	100%
1	11.33	0.001	13.3	851.86	15.7%
2	11.18	0.001	13.3	840.48	15.5%
3	12.93	0.001	13.3	972.16	17.9%
Average				888 ± 72	16 ± 1%

^aSee certificate of analysis in Data File S1.

^bFlag-MsrB1-His molecular weight: 14,579 g/mol

^cExpected Se concentration for 100% Sec occupancy of MsrB1: 78.96 g Se/mol / 14,579 g protein/mol = 5416 µg of Se/g of protein.

Table S2. Strains used in this study

Strain	Genotype	Reference
BY4741	<i>MATa his3Δ0 leu2Δ0 Δmet15 ura3Δ0</i>	(Winzeler and Davis 1997)
MaV203	<i>MATα, leu2-3,112, trp1-901, his3Δ200, ade2-101, gal4Δ, gal80Δ, SPAL10::URA3, GAL1::lacZ, HIS3UAS GAL1::HIS3@LYS2, can1R, cyh2R</i>	(Vidal et al. 1996a; Vidal et al. 1996b)
KHY1	<i>MATα, leu2-3,112, trp1-901, his3Δ200, ade2-101, gal4Δ, gal80Δ, SPAL10::URA3, GAL1::lacZ, his3@LYS2::KanMX, can1R, cyh2R</i>	

Table S3. Plasmids used in this study

Name	Plasmid-gene	Reference
pRS423		(Christianson et al. 1992)
pRS424		(Christianson et al. 1992)
pRS425		(Christianson et al. 1992)
pRS426		(Christianson et al. 1992)
pKH24	pRS425-MmSCL	This study
pKH29	pRS425-AsSelA	This study
pKH30	pRS425-AsSelD	This study
pKH31	pRS424-AsSelA	This study
pKH39	pRS424-MmSCL	This study
pKH47	pRS424-MmSCL/AsSelA	This study
pKH58	pRS424-GAL4	This study
pKH60	pRS424-gal4-R110Am	This study

pKH66	pRS425- <i>tS(AGA)D2</i>	This study
pKH71	pRS425- <i>tS(CUA)D2</i>	This study
pKH74	pRS425- <i>tU(CUA)1</i>	This study
pKH75	pRS426- <i>sfGFP-S2Am</i>	This study
pKH77	pRS425- <i>AsSelD/tU(CUA)1</i>	This study
pKH79	pRS424- <i>gal4-C11S</i>	This study
pKH80	pRS424- <i>gal4-C21S</i>	This study
pKH81	pRS424- <i>gal4-C11Am</i>	This study
pKH82	pRS424- <i>gal4-C21Am</i>	This study
pKH84	pRS425- <i>AsSelD/tU(CUA)2</i>	This study
pKH85	pRS423- <i>gal4-C21Am</i>	This study
pKH86	pRS425- <i>AsSelD/tU(CUA)3</i>	This study
pKH95	pRS423- <i>gal4-C11Am/C21Am</i>	This study
pKH97	pRS424- <i>gal4-C11S/C21S</i>	This study
pKH98	pRS423- <i>gal4-C11S/C21Am</i>	This study
pKH99	pRS423- <i>gal4-C11S</i>	This study
pKH100	pRS423- <i>gal4-C21S</i>	This study
pKH101	pRS423- <i>gal4-C11Am</i>	This study
pKH102	pRS423- <i>gal4-C11S/C21S</i>	This study
	pRS426- <i>ADH1p-Flag-hMsrB1(95TAG)-RPS20</i>	

Table S4. Oligonucleotides used in this study

Name	Sequence
ASR-F	GCAGGAAAGAACATGGGGTGTACAATATGGACTTCCTCTTTTC
ASR-R	CCTTTTGCTCACATGTCGGGATGCACCATGAGG
adh1-F	CGCGGATCCGGGTGTACAATATGGACTTCCTCTTTTC
rpl41b-R	CCGCTCGAGTCGGGATGCACCATGAGG
gal4a	ACAATCAACTGCAAGCCTCCTGAAAGATGA
gal4b	TACAAATCATCAAGCATTTTTTGCACATATAGGA
gal4c	AAAATGCTTGATGATTTGTAATGAGATTGAGGAGG
gal4d	CGCTCTAGAACTAGTGGATCCAATAATCAATCTCAATTACGTTCCG
fADH1-F	TTCCTGCAGCCCGGGGGATCCGGGATCGAAGAAATGATGGTAAAT G
fADH1-R	GGAGGCTTGCAGTTGATTGTATGCTTGGTATAGC
R110S-F	AGTCTCCACTGAAGCCAATGAATCTGTGACGGCATCTTTA
R110S-R	TAAAGATGCCGTCACAGATTCATTGGCTTCAGTGGAGACT
R110Am-F	GTCTCCACTGAAGCCAATAATCTGTGACGGCATCTTTATTACATT ATCTT
R110Am-R	AAGATAATGTGAATAAAGATGCCGTCACAGATTAGTTGGCTTCAGT GGAGAC
C11S-F	CGGCAAATATCGCTTGCTTGTTCGATAGAAGACAGTAG
C11S-R	CTACTGTCTTCTATCGAACAAGCAAGCGATATTTGCCG
C21S-F	TTCGGTTTTTCTTTGGAGCTCTTGAGCTTTTAAAGTCGG

C21S-R	CCGACTTAAAAAGCTCAAGAGCTCCAAAGAAAAACCGAA
C11Am-F	GCTTTTTAAGTCGGCAAATATCCTATGCTTGTTTCGATAGAAGACAG
C11Am-R	CTGTCTTCTATCGAACAAGCATAGGATATTTGCCGACTTAAAAAGC
C21Am-1	CACTTCGGTTTTTCTTTGGACTACTTGAGCTTTTTAAGTCGGCAAATA
C21Am-2	TATTTGCCGACTTAAAAAGCTCAAGTAGTCCAAAGAAAAACCGAA GTG
tSD2-F	GCAGCCCGGGGGATCCGGATCTTGATTTGATGGGACTTCCT
tSD2-R	TAGAACTAGTGGATCCGCGATAAGTCGTGATGCGGT
tScua-F	GTAAAGGCGAAAGATTCTAAATCTTTGGGCT
tScua-R	AGCCCAAAGATTTAGAATCTTTCGCCTTAAC
tSec-F	CTAAATCTTTTGGGCTTTGCCCGGGCAGGTTTCGATTCCTGCAGTTGT CGTTTTTACCAAAAATTTTCCTCAATC
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tSec4-F	GCCCAAAGATTTAGAATCTTTCGCCGCCACCAGACGGCATAGTTG CC-TCAATTTACAAGTAATTTGAATATTATTAGATAGT
U2	CGTACGCTGCAGGTCGAC
D2	ATCGATGAATTCGAGCTCG
his3-F	ATGACAGAGCAGAAAGCCCTAGTAAAGCGTATTACAAATGAAACC AAGATCGTACGCTGCAGGTCGAC
his3-R	CTACATAAGAACACCTTTGGTGGAGGGAACATCGTTGGTACCATTG GGCGAATCGATGAATTCGAGCTCG
his3-P	GAAGAATATACTAAAAAATGAGCAGGCAAGATAAACGAAGGCAA AGATGACAGAGCAGAAAGCCCTAG
his3-T	ACATGTATATATATCGTATGCTGCAGCTTTAAATAATCGGTGTCACT ACATAAGAACACCTTTGGTGGAG
kanMX-F	GAATCGCAGACCGATACCA
Lys2-R	TTTAAGTGACATCACCCGAAAAG
4936_op_F	CATCATCACCACCATCACTAAG
4936_op_R	TGTATATGAGATAGTTGATTGTATGC
MsrB1_PRS_F	TCAACTATCTCATATACAATGTCGTTCTGCAGCTTC
MsrB1_PRS_R	GTGATGGTGGTGGTGGTGGTACCCTGGGAGGCAG
FlagMsr_F	ATCTTAGGATCCATGGATTACAAGGATGACGACGATAAGATGTCGT TCTGCAGCTTC
FlagMsr_R	CGCGGAGGATCCTGTATATGAGATAGTTGATTGTATGCTTG

References

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