SUPPLEMENTAL DATA

Primers used for cloning

Primer	Sequence (5'-3')
At-CK2A1 5' NdeI	ATA*CATATG*TCGAAAGCTCGTGTGTACACCG
At-CK2A1 3' BamHI	AGT*GGATCC*TCATTGACTTCTCATTCTGCTGG
At-CK2A1 5' BspHI	AGCTAGCT*TCATGA*TGTCGAAAGCTCGTGTG
At-CK2A1 3' XhoI	GCT*CTCGAG*TTGACTTCTCATTCTGC
At-CK2A2 5' NcoI	ACTG*CCATGGG*TTCGAAAGCTCGTG
At-CK2A2 3' XhoI	AGCT*CTCGAG*TTGAGTCCTCATTCTGC
At-CK2A3 5' NdeI	CCA*CATATG*TCGAAAGCTAGGGTTTATAC
At-CK2A3 3' NotI	CGAGT*GCGGCCGC*CTGAGTTCGTAGTCTGCTGC
At-CK2Acp 5' NdeI	GACT*CATATG*GCCTTAAGGCCTTGTAC
At-CK2Acp 3' XhoI	TGTG*CTCGAG*CTGGCTGCGCGGCGTACGG
At-CK2B1 5' NcoI	ACTG*CCATGGG*TTATAGAGACAGAGG
At-CK2B1 3' XhoI	GGAC*CTCGAG*CGGTTTGTGTAATTTGAACC
At-CK2B2 5' NcoI	AGCT*CCATGG*GTTATAGGGAGAGAGGTA
At-CK2B2 3' XhoI	AGCT*CTCGAG*CGGCTTGTGTAGCTT
At-CK2B3 5' NcoI	AGCT*CCATGG*GTTACAAGGAACGTAGTGGA
At-CK2B3 3' XhoI	GAT*CTCGAG*TGGTTTGTGTACCTTGAAGCC
At-CK2B4 5' NcoI	GCAC*CCATGG*ATGTACAAGGATCGGAGTGG
At-CK2B4 3' XhoI	TGTG*CTCGAG*TTGTTTGTTGTGTACCTTAAAGCC
At-HD2B 5' NcoI	AGCTAGCT*CCATGG*AGTTCTGGGGAGTTGCGG
At-HD2B 3' XhoI	AGCT*CTCGAG*AGCTCTACCCTTTCCCTT
At-eIF2alpha 5'NcoI	ACGT*CCATGG*CGAATCCTGCTCCGAATCTAG
At-eIF2alpha 3'XhoI	GACT*CTCGAG*TTCAATTATCCCGCTACCTCC
Ta-eIF2alpha 5' NdeI	CAGC*CATATG*GCGAACCTCGAGTGC
Ta-eIF2alpha 3' BamEco	*GAATTCGGATCC*TTAGTCCGCATGGAC
At-eIF2beta 5'BamHI	ATGACTGAT*GGATCC*AAGGAGATATACATATGGCTGAT
At-eIF2beta 3'SalI	TGCTATGCT*GTCGAC*TCATTATTAAGTCTTCCTG
Ta-eIF3c 5' NcoI	AGCT*CCATGG*CGTCTCTGTTTTTGGGGAC
Ta-eIF3c 3' SacI	AGCT*GAGCTC*CTAATTTCTACCAGGCC
Ta-eIF3c 3' XhoI	AGCT*CTCGAG*ATTTCTACCAGGCCTG
At-eIF5 5' NcoI	ACTAGTACTGAT*CCATGG*CGCTGC
At-eIF5 3' BamHI	CAGATCAGT*GGATCC*TTATTAATCCTCCTCTTCGG

Subcellular localization of *A. thaliana* CK2 subunits. Salinas *et al*. have characterized the subcellular localization of all *A. thaliana* CK2 proteins by fusion protein expression (14). The Cell eFP Browser depicts data from the SUBA database (http://www.plantenergy.uwa.edu.au/suba2/) which contains both computationally predicted and experimentally documented subcellular localization data for *A. thaliana* proteins based on mass spectroscopic analysis of subcellular fractions and fluorescent fusion proteins (www.bar.utoronto.ca/).

Subunit		**Fusion Protein Expression**	**Cell eFP**
CK2α1	At5g67380	Nuclear, enriched in nucleolus	Mitochondrial and Cytoplasmic
CK2α2	At3g50000	Nuclear, enriched in nucleolus	Cytoplasmic
CK2α3	At2g23080	Nuclear	N/A
CK2αcp	At2g23070	Chloroplastic	Chloroplastic
CK2β1	At5g47080	Nuclear and Cytoplasmic	Nuclear
CK2β2	At4g17640	Nuclear	Nuclear
CK2β3	At3g60250	Nuclear and Cytoplasmic	Nuclear
CK2β4	At2g44680	Cytoplasmic	Nuclear and Cytoplasmic

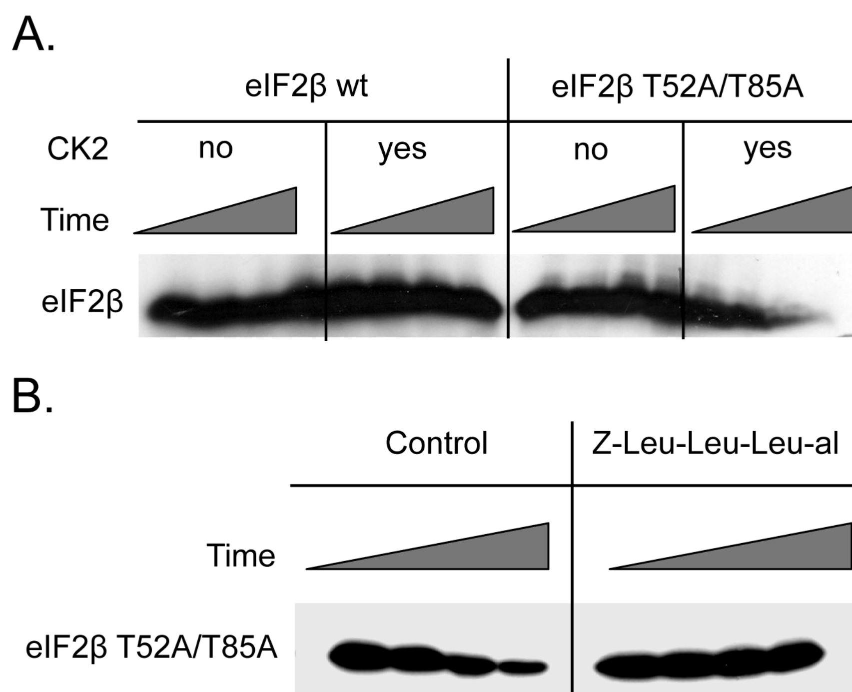

Figure Legend: CK2 phosphorylation sites may regulate the degradation of eIF2β. Panel A. Wild-type recombinant wheat eIF2β or eIF2β T52A/T85A (5 μg) was incubated with wheat germ S30 extract (150 μg) in the presence of 20 mM Hepes-KOH, pH 7.6, 5 mM $MgCl_2$, 0.2 mM ATP, 250 nM Calyculin A, and CK2α2β4 (100 ng) as indicated. Each 50 μl reaction was incubated at 37° C for 0-3 hrs and stopped with 1% SDS. 10 μl of each reaction was separated by 12.5% SDS-PAGE, transferred to PVDF, and analyzed with 1/5000 dilution of rabbit antisera to eIF2β. Panel B. A similar reaction was run in the presence and absence of the proteasome inhibitor Z-Leu-Leu-Leu-al (M132, Sigma). Recombinant wheat eIF2β T52A/T85A (5 μg) was incubated with wheat germ S30 extract (150 μg) in the presence of 20 mM Hepes-KOH, pH 7.6, 5 mM $MgCl_2$, 0.2 mM ATP, 250 nM Calyculin A, and CK2α2β4 (100 ng). Each 50 μl reaction was incubated at 37° C for 0-3 hrs and stopped with 1% SDS. 10 μl of each reaction was separated by 12.5% SDS-PAGE, transferred to PVDF, and analyzed with 1/5000 dilution of rabbit antisera to eIF2β.

Results:

In the presence of CK2, the phosphorylation of eIF2β may prevent its degradation upon exposure to wheat germ extract, as the non-phosphorylatable T52A/T85A mutant is degraded over time (panel A). A similar effect is not observed in the absence of exogenous CK2, nor with wild-type eIF2β suggesting that this does not appear to be a direct effect of phosphorylation. The degradation of eIF2β T52A/T85A was prevented by the proteasome inhibitor Z-Leu-Leu-Leu-al, (panel B), suggesting the degradation of recombinant eIF2β by wheat germ extract is taking place via the proteasome. These results suggest that the phosphorylation of eIF2β by CK2 may be related to protein stability through both direct and indirect means.