GRANT NUMBER: DE-FG02-04ER15575

“Protein Synthesis Initiation Factors: Phosphorylation and Regulation”

Karen S. Browning, University of Texas at Austin

1 University Station A5300

Department of Chemistry and Biochemistry

Austin, TX 78712

512-471-4562

kbrowning@mail.utexas.edu

DOE Program Office: Energy Biosciences

DOE Program Manager: Dr. Richard V. Greene

Data described in this report is in press and may be released.

**Final Technical Report
June 2004-present****Executive Summary**

The initiation of the synthesis of proteins is a fundamental process shared by all living organisms. Each organism has both shared and unique mechanisms for regulation of this vital process. Higher plants provide for a major amount of fixation of carbon from the environment and turn this carbon into food and fuel sources for our use. However, we have very little understanding of how plants regulate the synthesis of the proteins necessary for these metabolic processes. The research carried out during the grant period sought to address some of these unknowns in the regulation of protein synthesis initiation. Our first goal was to determine if phosphorylation plays a significant role in plant initiation of protein synthesis. The role of phosphorylation, although well documented in mammalian protein synthesis regulation, is not well studied in plants. We showed that several of the factors necessary for the initiation of protein synthesis were targets of plant casein kinase and showed differential phosphorylation by the plant specific isoforms of this kinase. In addition, we identified and confirmed the phosphorylation sites in five of the plant initiation factors. Further, we showed that phosphorylation of one of these factors, eIF5, affected the ability of the factor to participate in the initiation process. Our second goal was to develop a method to make initiation factor 3 (eIF3) using recombinant methods. To date, we successfully cloned and expressed 13/13 subunits of wheat eIF3 in *E. coli* using *de novo* gene construction methods. The final step in this process is to place the subunits into three different plasmid operons for co-expression. Successful completion of expression of eIF3 will be an invaluable tool to the plant translation community.

Comparison of Accomplishments and Goals.

There has been considerable progress made towards a better understanding of the role of phosphorylation of plant initiation factors by plant specific casein kinases and we are well on the way to completion of the construction of the eIF3 expression operon. These results are summarized below.

Specific Aim 1. Analysis of the phosphorylation states of initiation factors by mass spectrometry. The original goal to use mass spectrometry for identification of translation factor phosphopeptides in plant extracts was hampered by the slow arrival of a new FTICR mass spectrometer (purchased by department prior to grant period) and hiring of the personnel by the department mass spectrometry facility to run the instrument. We decided it would be more efficient and provide better information to focus on those initiation factors we knew were substrates for kinases and could manipulate for *in vitro* characterization. Consequently, we identified by routine mass spectrometry the kinase previously identified by this laboratory that use eIF2 and eIF3 as substrates (1). The kinase was shown to be a bona fide plant casein kinase 2. The casein kinase subunits from *Arabidopsis* were cloned, expressed and used to identify additional initiation factor substrates for characterization. This approach yielded functional casein kinase 2 from most of the *Arabidopsis* CK2 α and β genes, antibodies to *Arabidopsis* CK2 α and β subunits, expressed wheat eIF3c and antibody, expressed wheat eIF5 and antibody, expressed wheat eIF1 and antibody, and identification and biochemical confirmation of casein kinase sites in eIF2 α,β , eIF3c, eIF4B and eIF5. We have discovered new information about phosphorylation of translation initiation factors and generated valuable new tools that will be useful in dissecting plant initiation and its regulation. We have also shown that the plant multi-factor complex may be regulated by phosphorylation of eIF5, eIF3c and eIF2 α,β . These experiments were completed as the dissertation research for Michael Dennis (May 2008) and three publications are anticipated. Details of this work are presented below.

Specific Aim 2. Molecular analysis of the structure and function of the 11 subunits of eIF3. During the period of the grant two additional subunits for eIF3 were identified so that the complete eIF3 complex contains 13 subunits. The eIF3 complex construction and expression is being completed as part of the Freshman Research Initiative (FRI) research stream as described in detail below. All 13 subunits have been cloned using *de novo* gene synthesis and 12 have been shown to express in *E. coli*. Many of the subunits have been assembled into 2-subunit expression units and shown to express. If successful regeneration of the complete 13 subunit complex is obtained, then a publication including all undergraduate researchers involved is anticipated. A recombinant for eIF3 complex will be a valuable tool to the plant molecular community. Details of this work are presented below.

Summary of Objectives Achieved During Funding Period.

Specific Aim 1: Analysis of the phosphorylation states of initiation factors by mass spectrometry. Methods: two dimensional separation (HPLC or gel) of plant extracts; preparation of tryptic peptides; identification of phosphopeptides by mass spectrometry.

Changes from original plan: The original goal to use mass spectrometry for identification of translation factor phosphopeptides in plant extracts was hampered by the slow arrival of a new FTICR mass spectrometer (purchased by department prior to grant period) and delayed hiring of the personnel by the department mass spectrometry facility to run the instrument. We decided it would be more efficient and provide better information to focus on those initiation factors we knew were substrates for kinases and could manipulate for *in vitro* characterization. Consequently, we identified by routine mass spectrometry the kinase previously identified by this laboratory (1) that use eIF2 and eIF3 as substrates as a plant casein kinase 2.

Identification of wheat germ kinases. We had previously determined that a kinase from wheat germ used eIF2 α and eIF3c as substrates (1). Using mass spectrometry we identified the wheat kinase as wheat casein kinase 2 (CK2, [BAB59136](#)) and similar to *Arabidopsis* casein kinase 2 (At5g67380). We also identified by mass spectrometry the wheat casein kinase substrate reported by Yan *et al.* (2) as histone deacetylase (HD2 β).

We attempted to identify another wheat germ kinase previously reported (3) that uses eIF4G, eIFiso4G and eIF4B as substrates; however, although we were able to obtain peptides for mass spectrometry, there was not an unambiguous match in the existing databases. We will continue to try to match the protein as the wheat genome project progresses.

Figure 2 . Native Gel Electrophoresis of CK2 holoenzymes. 10 pmol of each CK2 homotetramer was analyzed using non-denaturing gel electrophoresis and Coomassie blue staining. Upon formation of the holoenzyme, CK2 subunits migrate as a single high molecular weight band. Lane 1. CK2 α 1 β 1; Lane 2. CK2 α 1 β 2; Lane 3. CK2 α 1 β 3; Lane 4. CK2 α 1 β 4; Lane 5. CK2 α 2 β 1; Lane 6. CK2 α 2 β 2; Lane 7. CK2 α 2 β 3; Lane 8. CK2 α 2 β 4.

Efforts were made to identify additional kinases from wheat germ. Extracts were fractionated on DEAE columns and assayed with a variety of substrates to look for kinases for subunits of eIF3, subunits of eIF4A, eIF4B, subunits of eIF4F, and subunits of eIFiso4F. The kinases identified turned

Figure 1: Gel Filtration of recombinant CK2 subunits and holoenzyme. CK2 α 1, CK2 β 1 and CK2 holoenzyme show distinct molecular weight differences when analyzed by gel filtration using S-200 HR at 150 mM KCl. Protein elution from the 3 separate column runs was monitored by A_{280} . CK2 α 1 (37.4 kDa) migrates as a monomer, while CK2 β 1 (33.4kDa) migrates as a dimer. When combined CK2 α 1 and β 1 elute as a single high molecular weight peak, indicating formation of the holoenzyme product. Elution volumes for molecular size markers are indicated with arrows.

out to be the same two kinases previously identified. Metabolically active tissues rather than wheat germ may be more appropriate to look for additional kinases for the translational apparatus. We decided to focus on the casein kinase 2 (CK2) and its role(s) in regulation of translation since it phosphorylated several initiation factors known to participate in the multi-factor complex during initiation of translation.

Substrate	CK2 Phosphorylation Sites
eIF2 α	S318
eIF2 β	T52, T85
eIF3c	S11, S13, S22, S26, S28, S31, S53, S56, S198, S210, S212, S215
eIF4B	S106, S140, S241
eIF5	S209, T240, S451

Table 1 . Confirmed CK2 phosphorylation of recombinant wheat translation initiation factors. Using MALDI MS/MS 20 *in vitro* CK2 phosphorylation sites have been identified in various recombinant wheat initiation factors. Most striking is the large number of phosphorylation sites (12) seen in the N-terminus of eIF3c.

Expression of *Arabidopsis* casein kinase 2 subunits.

In *Arabidopsis*, there are 4 genes for the catalytic α subunits and 4 genes for the regulatory β subunits of CK2 (4). We have cloned and over-expressed two of the four *Arabidopsis* CK2 α and all four CK2 β subunits. These subunits form stable complexes as indicated by gel filtration (Figure 1) and gel shift (Figure 2). The complexes are active against a variety of initiation factor substrates as shown in Table 1. Like native CK2 α subunits, recombinant *Arabidopsis* CK2 α subunits exhibit activity in the absence of regulatory CK2 β subunits although holoenzyme formation and the activity of the kinase can be modulated by the β -subunits (4-6). We also cloned and expressed *Arabidopsis* histone deacetylase HD2 β (At5g22650) and showed that it was a substrate for the recombinant CK2 complexes as previously reported for the native protein (2). In addition, antibodies have been raised for both α and β subunits of *Arabidopsis* CK2. These antibodies will be

Figure 3. Phosphorylation of Wheat Initiation Factors by CK2 Complexes. The activity of CK2 $\alpha 1$, CK2 $\alpha 2$, and the 8 complexes that form when each CK2 α subunit is combined in a 1:1 molar ratio with each of the 4 CK2 β subunits ($\beta 1$, $\beta 2$, $\beta 3$, $\beta 4$) was evaluated by [32 P]-ATP incorporation into the recombinant substrate. Each sample was incubated with kinase at 25°C for 15 minutes, the reaction was stopped by the addition of 4xSDS loading dye, the samples were run on a gel, and exposed to a phosphorimager screen. The phosphorylation of each substrate was run in triplicate and quantitated by densitometry (BioRad).

useful for immuno-precipitation to identify additional substrates of CK2 and for preparation of extracts depleted of CK2.

Identification of Translation Factors Substrates of CK2. In addition to eIF2 α and eIF3c previously identified as substrates, eIF2 β , eIF5 and eIF4B were also found to be substrates of CK2 (see Table 1).

eIF3c. eIF3c has 15 predicted CK2 consensus sites (www.scansite.mit.edu, cut-off score 0.59). We identified 12 CK2 phosphorylation sites using recombinant kinase to

Figure 4. Phosphorylation sites of wheat eIF3c and eIF5. Wheat eIF3c contains 15 predicted CK2 phosphorylation sites which Scansite (www.scansite.mit.edu) identifies having a score below 0.59. Using mass spectrometry, 12 of these sites have been confirmed by *in vitro* CK2 phosphorylation. Diamonds represent phosphorylation sites detected by mass spectrometry; consensus sites not detected are depicted as circles. The NTD of eIF3c has been previously shown to play an important role in binding to eIF1 and eIF5 in the yeast MFC.

phosphorylate eIF3c *in vitro* followed by identification using mass spectrometry of the tryptic phosphopeptides (see Fig. 4). The N-terminal region of eIF3c is highly acidic (aspartic and glutamic acid residues) and when phosphorylated it is difficult to analyze using mass spectrometry; however, expression of only the N-terminal 315 amino acids of eIF3c allowed identification of the phosphorylation sites by enriching for phosphopeptides in this region.

Analysis of the phosphorylation of recombinant eIF3c and in native complex by CK2 isoforms is shown in Figure 3 A and B. Different combinations of α and/or β subunits showed differential activity with the two substrates, suggesting that there is specificity of CK2 isoforms. As expected, the recombinant eIF3c showed higher levels of phosphorylation than the native eIF3c in complex since at least some of the sites were likely already phosphorylated or unavailable in the native complex.

eIF5. eIF5 has 3 predicted CK2 consensus sites. We have identified three distinct CK2 *in vitro* phosphorylation sites in recombinant eIF5: S209, T240, and S451 (see Fig. 4). S451 is located in the C-terminal HEAT domain involved in interaction with eIF1, eIF2, and eIF3 (7). Various

Fig. 5. eIF5-dependant translation assay
The ability of recombinant wheat eIF5 to stimulate translation in eIF5-depleted wheat germ S30 extracts was analyzed. Each 50 μ l reaction contained 20 μ l of eIF5 depleted wheat germ extract, 5 pmol HSP21 mRNA template, and recombinant wheat eIF5, eIF5 S451E, eIF5 S451A, or eIF5AAA as indicated.

mutants containing alanine or glutamate substitutions at the various phosphorylation sites have been cloned and expressed. Preliminary binding experiments suggest that alanine substitution at these phosphorylation sites leads to a reduction in eIF2 binding via eIF2 β in gel shift analysis. In addition, an eIF5 dependent assay was developed and mutations of the phosphorylation sites were demonstrated to have an effect on translational activity (Fig. 5). Immuno-affinity purified native wheat eIF5 analyzed by mass spectrometry confirmed the existence of these phosphorylation sites *in vivo*. The phosphorylation pattern by CK2 isoforms is shown in Figure 3C and indicates some preference for certain β subunits; however the preference is not as striking as that observed for eIF2 β (compare Fig. 3C and Fig. 3E).

Figure 6. Multifactor complex (MFC) interactions between eIF1, eIF2, eIF3, and eIF5. Eukaryotic translation initiation factors eIF1, the eIF2-met-tRNA ternary complex, eIF3, and eIF5 can pre-assemble into a multifactor complex (MFC). These factors can then be simultaneously loaded onto the 40S ribosomal subunit in combination with eIF1A to form the 43S pre-initiation complex (PIC). Within the MFC each factor appears to interact with all three of the other factors. A number of the binding points between factors in the MFC are based on electrostatic interactions, and a number of these interaction points are phosphorylated by CK2. We believe that CK2 may play an important role in regulating the formation of the MFC through phosphorylation of these initiation factors.

eIF4B. We have identified 3 phosphorylation sites *in vitro*. Recombinant wheat eIF4B is fully phosphorylated by CK2 on S140 and partially phosphorylated on residues S106 and S241. Confirmation of these sites by mutagenesis is in progress. CK2 isoforms show no specificity for eIF4B (Fig. 3D).

eIF2 α . eIF2 α has 3 predicted CK2 consensus sites; however, only S318 is of high stringency. S318 was identified by mass spectrometry as the only CK2 *in vitro* phosphorylation site. This was then confirmed by site-directed mutagenesis, as S318A or S318E of S318 were no longer the Ser51 involved in regulation of mammalian eIF2 α . **It is not clear that plants use phosphorylation of eIF2 α Ser51 for regulation since comparable kinases (e.g. PKR) have not been isolated from plants yet.** The S318 site is similar however in location to three CK2 phosphorylation sites in the C-terminus of

yeast eIF2 α (8). There is no site similar to S318 in mammalian eIF2 α , and mammalian CK2 only phosphorylates eIF2 β and not eIF2 α (9). The function of these C-terminal phosphorylation sites in yeast and plants has yet to be determined. As shown in Figure 3F, all combinations of CK2 subunits phosphorylate S318 and do not display any specificity for this substrate similar to that observed for eIF4B.

eIF2 β . Recombinant eIF2 β was found to have 2 *in vitro* phosphorylation sites, which required the presence of CK2 β for phosphorylation (Fig. 3E) suggesting a high degree of specificity of the substrate for the holoenzyme and a possible target for regulation. The peptide 46TEGLSVTESGEASFVGLK63 contains a single phosphorylation site; unfortunately, the MS/MS spectra for this region could not differentiate between the two potential CK2 sites, T52 and S54. In addition, T85 was also observed to be partially phosphorylated in mass spectrometry. Site directed mutagenesis confirmed T52 and T85 as the correct phosphorylation sites.

Formation of multi-factor complex (MFC). Initiation factors involved in binding to the 40S ribosome (eIF3, eIF5, eIF1, and the eIF2 ternary complex) form what is called the multi-factor complex (MFC, 10-12). This MFC binds to eIF1A and the 40S ribosome to form the 43S pre-initiation complex (PIC), which subsequently binds to the eIF4s-mRNA complex and then binds the 60S ribosome to form a functional 80S ribosome. It

Figure 7. Phosphorylation of recombinant wheat His-eIF5 by CK2 enhances its interaction with eIF1. Alanine mutations at CK2 phosphorylation sites prevent the CK2 mediated increase in eIF1 binding to eIF5.

is striking that 3 of the 4 plant factors involved in the yeast MFC are all substrates for plant CK2. eIF1 does not appear to be phosphorylated by CK2, nor does it contain any predicted phosphorylation sites for CK2 or other protein kinases. A diagram of the interaction between these initiation factors in the yeast MFC is shown in Figure 6. eIF5 plays a central role in the assembly of the yeast MFC, as its C-terminus binds to eIF1, the K-boxes (lysine rich regions in the N-terminus) of eIF2 β , and the N-terminal domain

(NTD) of eIF3c simultaneously (7; 13; 14). eIF1 binds to both the NTD of eIF3c and the C-terminal domain (CTD) of eIF5 (14). Interestingly, CK2 phosphorylates both the N-terminus of eIF3c and the C-terminus of eIF5, at critical binding points for formation of the MFC (Fig. 4). Public DNA array data indicates that different CK2 subunits are differentially expressed and consequently could play a role in regulation of the formation of the multi-factor complex at different times in development or during environmental stresses. Using pull down experiments with purified factors and kinases (see Fig. 7), we obtained evidence that phosphorylation of eIF5 increases the ability to form the multi-factor complex and that eIF5 mutants that cannot be phosphorylated are less active in translation (see Fig. 5). **These results suggest that the phosphorylation state of at least one initiation factor plays a role in the efficiency of translation initiation.**

Manuscripts Accepted for Publication:

1. Dennis, M.D. and Browning, K.S. **Differential phosphorylation of plant translation factors by *Arabidopsis thaliana* CK2 holoenzymes**, *J. Biological Chemistry*, in press
2. Dennis, M.D., Person, M. and Browning, K.S. **Phosphorylation of plant translation initiation factors by CK2 enhances the *in vitro* interaction of multifactor complex components**, *J. Biological Chemistry*, in press.
3. Mayberry, L.K., Allen, M.L., Dennis, M.D. and Browning, K.S. **Evidence for variation in the optimal translation initiation complex: Plant eIF4B, eIF4F and eIF(iso)4F Differentially Promote Translation of mRNAs**, *Plant Physiology*, in press

Specific Aim 2: Molecular analysis of the structure and function of the 11 subunits of eIF3. Methods: over-expression of subunits and assembly of recombinant complex for biochemical analysis and mutagenesis; T-DNA analysis of genes for subunits or gene silencing; DNA array analysis of expression of eIF3 subunits.

Changes from original plan: The number of subunits in the complex increased to 13 from 11. The method of cloning the eIF3 subunits was changed from standard cDNA to *de novo* gene synthesis. T-DNA analysis and DNA array analysis will be carried out as part of an *Arabidopsis* 2010 initiative (if funded) or in collaboration with Albrecht von Arnim (University of Tennessee-Knoxville).

Cloning of subunits of eIF3. Recently new subunits, eIF3l and eIF3m, for mammalian (15; 16) and *S. pombe* eIF3 (17) were identified. eIF3l had been previously identified as “plant specific” (18). What would be plant eIF3m was identified as “unknown” in Burks *et al.* (18) and mass spectrometry of the plant peptides (Burks, Browning, unpublished data) indicates similarity to the eIF3m identified in both *S. pombe* and mammals (16; 17). Recently a homolog for the mammalian eIF3j was identified in plants.

We conventionally cloned 11/13 subunits of both wheat and *Arabidopsis* eIF3 subunits from ESTs or cDNAs from the ABRC or the wheat EST projects in preparation for making the operon. However, during the project period the PI became involved in the iGEM synthetic biology team for UT (http://parts2.mit.edu/wiki/index.php/Main_Page) and discovered the BioBrick method for making “parts” to make biological circuits. BioBricks are assembled *de novo* from oligonucleotides that are designed to optimize codon expression for a protein in the desired organism and to eliminate undesirable restriction sites (<http://parts.mit.edu/registry/index.php/Help:Contents>). BioBricks are all assembled using the same sets of restriction sites. This method seemed far more pliable to reaching the desired goal of having all 13 subunits of plant eIF3 expressed in *E. coli* than struggling with restriction sites and potential issues of codon usage. However, when presented with this idea, post-doctoral and graduate students were very reluctant to take on such a large cloning project that also runs a high risk of failure in the end. It is possible that one or more of the subunits will fail to assemble *in vivo* in *E. coli* either due to expression issues or possible post-translational modifications necessary for assembly. The PI also became involved with a new program at UT called the Freshman Research Initiative (FRI). This program teaches freshmen their first semester about the basics of research. In the second semester they are placed into “research streams” in groups of 25-30 students lead by a “research educator”. The research educator is a Ph.D. level scientist that assists the students in carrying out a PI’s research project. I chose making of eIF3

Figure 6. BioBrick assembly taken from <http://parts.mit.edu/registry/index.php/Help:Contents>. All bricks are made with the EcoRI(E)/XbaI(X) prefix and the SpeI(S)/PstI(P) suffix. When assembled in this manner the prefix and suffix are regenerated every time a brick part is added.

BioBricks to be the topic of my stream. This is an ideal project, the methods are simple enough (PCR, cloning, screening, subcloning, expression) for freshman to learn the skills needed and it has many “parts” so that each pair of students gets a “unique” aspect of the project to work on. We started this project in Spring 2007 and it has been enormously successful. We have acquired 12/13 complete subunits that have been sequenced and are correct. The remaining subunit eIF3j is in progress to due its late identification. We fully anticipate that we will complete the cloning of all the subunit bricks by the end of Fall 2008. The FRI stream students have confirmed individual subunit expression in *E. coli* using monoclonal antibodies to most of the subunits. The subunits are being assembled into multiple groups for the assembly into an operon for expression of the complex. So far, various combinations of up to 4 subunits also express protein. All 3 plasmids with operons containing the subunits are ready for co-expression of the complex during summer 2009 and if successful will be published. **Recombinant eIF3 complex will be an invaluable tool for the study of plant eIF3 assembly and function and will greatly benefit the plant molecular community.**

Other laboratory publications during grant period:

Gazo, B.G., Murphy, P., Gatchel, J.R. and **Browning, K.S.** (2004) A novel interaction of cap-binding protein complexes eIF4F and eIF(iso)4F with a region in the 3' untranslated region of Satellite Tobacco Necrosis Virus. *J. Biol. Chem.* **279**: 13584-92.

Park, H.S., **Browning, K.S.**, Hohn, T., and Ryabova, L.A. (2004) Eucaryotic initiation factor 4B controls eIF3-mediated ribosomal entry of viral reinitiation factor. *EMBO J.* **23**: 1381-91.

Pavel, I., Belcher, A. and **Browning, K.S.** (2004) A Method for Coupled Transcription and Aminoacylation of cys-tRNA. *Anal. Biochem* **335**: 192-195.

Browning, K.S. (2004) Plant translation initiation factors: it is not easy to be green. *Biochem. Soc. Trans.* (2004) **32**: 589–591.

Mayberry, L. and **Browning, K.S.** (2006) In vitro translation of plant viral RNA. *Current Protocols in Microbiology*, pp. 16K1.1-16K1.12. Wiley, New York.

Nicaise, V., Gallois, J.L., Chafiai, F., Allen, M.L., Schurdi-Levraud, V., **Browning, K.S.**, Candresse, T., Caranta, C., Le Gall, O., German-Retana, S. (2007) Coordinated and selective recruitment of eIF4E and eIF4G factors for potyvirus infection in *Arabidopsis thaliana* FEBS Lett. 581: 1041-1046.

Mayberry, L.K., Dennis, M.D., Allen, M.L., Nitka, K.A., Murphy, P.A., Campbell, L. and **Browning, K.S.** (2007) Expression and purification of recombinant wheat translation initiation factors eIF1, eIF1A, eIF4A, eIF4B, eIF4F, eIF(iso)4F and eIF5. *Meth. Enzymol.* 430:397-408.

Monzingo, A.F., Dhaliwal, S., Dutt-Chaudhuri, A., Lyon, A., Sadow, J., Hoffman, D.W., Robertus, J.D., and **Browning, K.S.** (2007) The Structure of Translation Initiation Factor eIF4E from Wheat Reveals a Novel Disulfide Bond. *Plant Phys.* 143: 1504-1518.

Treder, K., Kneller, E.L., Allen, E.M., Wang, Z., **Browning, K.S.**, and Miller, W.A. (2007) The 3' Cap-Independent Translation Element of Barley Yellow Dwarf Virus Binds eIF4F via the eIF4G Subunit to Initiate Translation. *RNA* 14: 134-47.

Pierrat, O.A., Mikitova, V., Bush, M.S., **Browning, K.S.**, Doonan, J.H. (2007) Control of protein translation by phosphorylation of the mRNA 5'-cap-binding complex. *Biochem Soc Trans.* 35: 1634-47.

References Cited

1. Browning, K. S., Yan, T. F. J., Lauer, S. J., Aquino, L. A., Tao, M., and Ravel, J. M. Phosphorylation of wheat germ initiation factors and ribosomal proteins. *Plant Physiology* 77, 370-373. 1985.
2. Yan, T. F. and Tao, M. Studies on an endogenous substrate of wheat germ protein kinase. *J.Biol Chem.* 257[12], 7044-7049. 6-25-1982.
3. Humphreys, J., Browning, K. S., and Ravel, J. M. Identification of a Kinase in Wheat Germ That Phosphorylates the Large Subunit of Initiation Factor 4F. *Plant Physiology* 88, 483-486. 1988.
4. Salinas, P., Fuentes, D., Vidal, E., Jordana, X., Echeverria, M., and Holuigue, L. An extensive survey of CK2 alpha and beta subunits in Arabidopsis: Multiple isoforms exhibit differential subcellular localization. *Plant and Cell Physiology* 47[9], 1295-1308. 2006.
5. Mizoguchi, T., Yamaguchi-Shinozaki, K., Hayashida, N., Kamada, H., and Shinozaki, K. Cloning and characterization of two cDNAs encoding casein kinase II catalytic subunits in Arabidopsis thaliana. *Plant Mol Biol* 21[2], 279-289. 1993.
6. Riera, M., Peracchia, G., and Pages, M. Distinctive features of plant protein kinase CK2. *Mol Cell Biochem* 227[1-2], 119-127. 2001.
7. Yamamoto, Y., Singh, C. R., Marintchev, A., Hall, N. S., Hannig, E. M., Wagner, G., and Asano, K. The eukaryotic initiation factor (eIF) 5 HEAT domain mediates multifactor assembly and scanning with distinct interfaces to eIF1, eIF2, eIF3, and eIF4G. *Proc.Natl.Acad.Sci.U.S.A* 102[45], 16164-16169. 11-8-2005.
8. Feng, L., Yoon, H. J., and Donahue, T. F. Casein Kinase-II Mediates Multiple Phosphorylation of Saccharomyces-Cerevisiae Eif-2-Alpha (Encoded by Sui2), Which Is Required for Optimal Eif-2 Function in Saccharomyces-Cerevisiae. *Molecular and Cellular Biology* 14[8], 5139-5153. 1994.
9. Pinna, L. A. Casein kinase 2: an 'eminence grise' in cellular regulation? *Biochim.Biophys.Acta* 1054[3], 267-284. 9-24-1990.

10. Asano, K., Clayton, J., Shalev, A., and Hinnebusch, A. G. A multifactor complex of eukaryotic initiation factors, eIF1, eIF2, eIF3, eIF5, and initiator tRNA^{Met} is an important translation initiation intermediate in vivo. *Genes and Development* 14[19], 2534-2546. 2000.
11. Asano, K., Phan, L., Valásek, L., Schoenfeld, L. W., Shalev, A., Clayton, J., Nielsen, K., Donahue, T. F., and Hinnebusch, A. G. A multifactor complex of eIF1, eIF2, eIF3, eIF5, and tRNA_i^{Met} promotes initiation complex assembly and couples GTP hydrolysis to AUG recognition. *Cold Spring Harbor Symposia on Quantitative Biology* 66, 403-415. 2001.
12. Asano, K. and Sachs, M. S. Translation factor control of ribosome conformation during start codon selection. *Genes Dev.* 21[11], 1280-1287. 6-1-2007.
13. Singh, C. R., Yamamoto, Y., and Asano, K. Physical association of eukaryotic initiation factor (eIF) 5 carboxyl-terminal domain with the lysine-rich eIF2beta segment strongly enhances its binding to eIF3. *J Biol.Chem.* 279[48], 49644-49655. 11-26-2004.
14. Singh, C. R., He, H., Li, M., Yamamoto, Y., and Asano, K. Efficient incorporation of eukaryotic initiation factor 1 into the multifactor complex is critical for formation of functional ribosomal preinitiation complexes in vivo. *J Biol.Chem.* 279[30], 31910-31920. 7-23-2004.
15. Morris-Desbois, C., Réty, S., Ferro, M., Garin, J., and Jalinot, P. The human protein HSPC021 interacts with Int-6 and is associated with eukaryotic translation initiation factor 3. *Journal of Biological Chemistry* 276[49 PT 1], 45988-45995. 2001.
16. Unbehaun, A., Borukhov, S. I., Hellen, C. U., and Pestova, T. V. Release of initiation factors from 48S complexes during ribosomal subunit joining and the link between establishment of codon-anticodon base-pairing and hydrolysis of eIF2-bound GTP. *Genes Dev.* 18[24], 3078-3093. 12-15-2004.
17. Zhou, Chunshui, Arslan, Fatih, Wee, Susan, Krishnan, Srinivasan, Ivanov, Alexander, Oliva, Anna, Leatherwood, Janet, and Wolf, Dieter. PCI proteins eIF3e and eIF3m define distinct translation initiation factor 3 complexes. *BMC Biology* 3[1], 14. 2005.
18. Burks, E. A., Bezerra, P. P., Le, H., Gallie, D. R., and Browning, K. S. Plant initiation factor 3 subunit composition resembles mammalian initiation factor 3 and has a novel subunit. *Journal of Biological Chemistry* 276[3], 2133-2131. 2001.