The eIF-2α Protein Kinases, Regulators of Translation in Eukaryotes from Yeasts to Humans*

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Covalent modification of translational machinery components by phosphorylation is a principal means of regulating protein synthesis in eukaryotic cells (1). Initiation of mRNA translation is a slow step in the protein synthesis pathway (2-5). A key protein factor required for translation initiation is eIF-2. Phosphorylation of the α subunit of protein synthesis initiation factor eIF-2 (eIF-2α) is one of the best characterized translational control mechanisms, both in lower and higher eukaryotes. The phosphorylation of eIF-2α on serine 51 leads to an inhibition of translation (2, 4).

Protein kinases that catalyze the phosphorylation of eIF-2α have been identified and characterized from a variety of eukaryotes, ranging from yeast to human cells. The eIF-2α kinases are cAMP-independent enzymes whose induction and activation are finely regulated (Fig. 1). Dependent upon the type of cell, stimuli ranging from cytokine treatment and viral infection to the availability of metabolites and cofactors required for cell function may modulate eIF-2α kinase activity (2, 4, 6-10). This review examines what is now known about three kinds of eIF-2α protein kinases: the RNA-dependent eIF-2α protein kinases (PKR) from human and mouse cells, PKR-Hum and PKR-Mus, respectively; HRI, the hemin-regulated eIF-2α protein kinase from rabbit reticulocytes; and GCN2, the general control eIF-2α protein kinase from yeast regulated by amino acid availability.

Structural Features of the eIF-2α Protein Kinases

The organization of the PKR, HRI, and GCN2 eIF-2α protein kinases is compared in Fig. 2. The catalytic subdomains characteristic of all eukaryotic protein serine/threonine kinases (11) are highly conserved among the different eIF-2α kinases. The catalytic cores of PKR-Hum, HRI, and GCN2 display 36-42% sequence identity in pairwise comparisons (12-17). The catalytic cores of the two PKR enzymes, PKR-Hum and PKR-Mus, are more closely related (12, 13, 18-20); PKR-Hum and PKR-Mus possess an overall amino acid identity of 62% and similarity of 75% (13).

The amino acid residue positions of four catalytic subdomains of the eIF-2α protein kinases, based on homology with other protein serine/threonine kinases (11), are summarized in Table I. Subdomains I, VI, and VII are involved in ATP binding and hydroxy amino acid recognition, and the subdomain II invariant lysine residue is central to the phosphotransfer reaction (11). The HRI and GCN2 eIF-2α kinases possess relatively large inserts between some of the catalytic subdomains, most notably between subdomains IV and VI, relative to other protein serine/threonine kinases including PKR. Therefore, the catalytic regions of the HRI and GCN2 kinases have a larger overall size than those of the PKR-Hum and PKR-Mus enzymes (Fig. 2). In contrast to the catalytic regions of the eIF-2α kinases, the noncatalytic regions of the proteins differ significantly between PKR, HRI, and GCN2. Furthermore, the GCN2 kinase is a significantly larger protein than the PKR and HRI kinases, in part because of the larger regulatory region of HRI, which shows significant homology with the histidyl-tRNA synthetase domains (15-17). Finally, the PKR, HRI, and GCN2 protein kinases are all phosphoproteins (6-10, 12-21).

PKR—Molecular cDNA clones of PKR have been isolated from human Daudi (12) and U (13) cells, and from mouse pre-B 70Z/3 (15) and PMA (19) cells. The human PKR is a 551-amino acid protein with a molecular mass of about 65 kDa as deduced from the cDNA ORF (12, 13). By contrast, the mouse PKR is a somewhat smaller protein of 518 amino acids with a molecular mass of about 59 kDa as deduced from the cDNA ORF (18). PKR is a ribosome-associated protein that can be dissociated from ribosomes by washing with high salt buffers (6, 13, 22). The sizes of the PKR cDNAs, about 2.4 kb, are comparable with the size of
of the major mRNA detected by Northern analysis, about 2.5 kb (12, 13, 18).

The conserved catalytic subdomains characteristic of protein serine/threonine kinases are, without exception, located in the C-terminal half of the PKR eIF-2α kinases (12, 13, 18). PKR kinases are activated by an RNA-dependent autophosphorylation (6), in which the activation of the subdomain II invariant lysine residue 296 with arginine (K296R) eliminates autophosphorylation activity and eIF-2α kinase activity associated with PKR (23–25). The N-terminal region of the PKR enzymes constitutes the regulatory domain (Fig. 2) and possesses the RNA binding activity (19, 23, 26, 27). The multiple sites of phosphorylation on PKR-Hum (22) and PKR-Mus (18, 22) proteins, predominantly serine residues but also including threonine, have not yet been identified. No PKR phosphorylation on tyrosine is detectable (18, 22).

The RNA binding activity of PKR maps to a region that includes a repeated subdomain, R, the core of which is about 20 amino acid residues (26). This core sequence is conserved in several other proteins now known to be RNA binding proteins and represents a new kind of RNA binding domain (26, 28). The core consensus motif is G-X-G-X-S/T-K-X-X-A-K-X-X-A-X-A-Hydrophobic X-X-L. Although multiple copies of the R motif are found in many of the RNA binding proteins including PKR-Hum, PKR-Mus, TRBP-Hum, Stauken-Dm, and RBPA-X1, only one copy is found in others including NS34-Rot, E3L-Vv, PAC1-Ysc, and RNase III-Ec. Furthermore, the DNA analysis of PKR has established that the N-terminal proximal copy of R is both necessary and sufficient for RNA binding activity (26, 29). However, both copies of R appear required for optimal RNA binding activity (19, 26, 27, 29). The significance of the fact that the two R region motifs of PKR appear to function as non-equivalent units is not yet clear.

**HRI**—The rabbit HRI kinase is a 626-amino acid protein with a molecular mass of about 70 kDa as deduced from the cDNA (14), although the apparent M, by gel electrophoresis is about 90 kDa (Table I). The size of the HRI cDNA, about 2.7 kb (14), is in good agreement with the size of the single mRNA species detected by Northern analysis, about 2.8 kb (30).

The HRI kinase possesses all 11 of the conserved catalytic subdomains that are characteristic features of protein serine/threonine kinases (14). Of particular uniqueness, however, is the insertion of about 140 amino acids between catalytic subdomains V and VI. The significance of this insert has not yet been established. HRI is present in heme-supplemented reticulocyte lysates as an inactive proinhibitor (10). The binding of heme directly to purified heme-reversible HRI has been demonstrated. HRI is activated as an eIF-2α kinase by heme deficiency; the activation of HRI is accompanied by its autophosphorylation (31). The S51A mutant of HRI appears to function as a non-equivalent unit.

**GCN2**—Two regions of the deduced 1590-amino acid sequence of the GCN2 protein display striking homology with known enzymes. (11) GCN2 protein is not able to carry out either the uridylation reaction or the phosphorylation of eIF-2α in vitro (21, 32). The adjacent H irS-R-like domain of the GCN2 protein is dispensable for the catalytic function of the protein kinase domain in vitro, but the H irS-R domain is required for positive regulatory functions of GCN2 in vivo (15, 32). Finally, the GCN2 protein is associated with ribosomes, possibly the 60 S subunit, and can be dissociated by washing with buffers containing 0.5 m KCl. The extreme C-terminal region of the GCN2 protein appears essential for its interaction with ribosomes (33).

### Table I: eIF-2α protein kinases

| Kinase* (accession no.) | Amino acids | Molecular mass, SDS-PAGE, cDNA ORF* | mRNA^ | Kinase catalytic subdomains^ |
|-------------------------|-------------|-----------------------------------|-------|-----------------------------|
| PKR-Hum (M65204)        | 551         | ~68 kDa                           | 2.6, 6.0 | I DLKFPN FPG               |
| PKR-Mus (M65209)        | 518         | ~65 kDa                           | 2.5, 4.0, 6.0 | II DLKPRN DFG              |
| HRI-Rab (M69035)        | 626         | ~90 kDa                           | 2.8    | I DLKFPN FPG               |
| GCN2-Ysc (M27082)       | 1,590       | ~180 kDa                          | 4.5    | I DLKFPN FPG               |

* PKR, the interferon-inducible, RNA-dependent eIF-2α protein kinase from human (PKR-Hum) and mouse (PKR-Mus) cells; HRI-Rab, the hemin-regulated eIF-2α kinase from rabbit reticulocytes; GCN2-Ysc, the eIF-2α kinase from the yeast *S. cerevisiae*. The most recent GenBank™/EMBL data bank accession numbers are provided based on nucleotide sequences reported for PKR-Hum (12, 15), PKR-Mus (18), HRI-Rab (14), and GCN2-Ysc (15, 16). Characteristics of each of the four eIF-2α protein kinases are shown in the remaining columns of the table.

^ The number of amino acids of the kinase as deduced from the cDNA ORF.

* The apparent molecular mass (kDa) of the kinase estimated by SDS-polyacrylamide gel electrophoresis (PAGE) and the theoretical size of the kinase calculated from the longest ORF of the cDNA.

^ The size (kb) of the mRNAs detected by Northern gel blot analysis, with the underlined value representing the major RNA species.

* The respective amino acid position of selected catalytic subdomains conserved among protein serine/threonine kinases (11).
around subdomains IX and X of the PKR, HRI, and GCN2 kinases that do not phosphorylate eIF-2α (13, 14, 17). These regions may be involved in aspects of substrate binding and phosphorylation unique to the α subunit of eIF-2. A synthetic peptide, P-74, which contains residues conserved in subdomain IX, inhibits the eIF-2α kinase activity of HRI (30).

**Regulation of the eIF-2α Protein Kinases**

Inactivation of initiation factor eIF-2 function by serine 51 phosphorylation is an important translation control mechanism, both for total cellular protein synthesis as illustrated by HRI in reticulocytes (10) and for gene-selective translational control as illustrated by GCN2 in yeast (41, 42) and PKR in mammalian cells (8, 43, 44). Regulation of the level of eIF-2α protein kinase activity occurs by several different mechanisms.

**PKR—PKR is regulated at the translational level by interferon treatment, as measured by both Northern gel blot and nuclear run-on analyses (6, 9, 12, 13). IFN-α is an efficient inducer of both the 2.5- and 6.0-kb mRNAs in human cells (12, 13), but IFN-γ is a poor inducer (13). Tissue-specific differences in the ratios of the three PKR transcripts are observed in mice; for example, the 2.5-kb mRNA is the predominant species in testes, but in both lung and heart tissues the 4.0-kb species is predominant (18). Transcription of PKR is not rapidly down-regulated, in contrast to some other IFN-inducible genes (12). However, the synthesis of PKR in transfected mammalian cells is autoregulated primarily at the level of translation by a mechanism that is likely dependent upon catalytically active PKR (25).

Activation of PKR is RNA-dependent but CAMP-independent (6, 8, 9, 45, 46, 48). A number of effectors of the RNA-dependent activation of PKR have been identified (Fig. 3). Activators include synthetic and natural double-stranded RNA, for example (25), and reovirus genome dsRNA, respectively. Common single-stranded RNA species are also activators, for example human immunodeficiency virus (HIV) TAR RNA and reovirus sRNA. Inhibitors of the autophosphorylation activation of PKR include dsRNA at high concentration and three highly structured ssRNA species: adenovirus VAI RNA, Epstein-Barr virus EBER RNA, and HIV TAR RNA. RNA binding proteins that sequester activator RNAs, for example the reovirus s3 protein and HIV TAR RNA, also activate the PKR activation process (48). In addition, 2-amino purine is a potent inhibitor of PKR, although cellular protein kinases in general are not inhibited by the purine analog (48). Some of the most direct evidence for the involvement of PKR in translational repression in mammalian cells emerges from the analysis of various RNA, protein, and purine inhibitors of kinase activity (8, 39, 46, 48).

The basis of the RNA selectivity of PKR activation is an important question. Kinase activation is associated with the formation of a stable PKR-dsRNA complex that requires at least 30-50 bp of duplex and is optimal with about 80 bp, although the PKR protein appears to interact with as little as 11 bp of dsRNA (47). Reovirus s1 RNA, a reovirus sRNA, and reverse virus genome dsRNA all bind to the same R region of the PKR protein (23, 26). Mutations in PKR that impair RNA binding have similar effects on the binding of both activator and inhibitor RNAs (29). This suggests that the discrimination between activator and inhibitor RNAs presumably takes place subsequent to RNA binding.

Little is known concerning the potential association of PKR with itself or with other proteins that may regulate PKR localization to the ribosome and activity. Cell fractionation studies reveal that PKR is cytosol-associated (6, 13, 22); in situ immunofluorescent staining studies reveal the PKR largely localized to the rough endoplasmic reticulum (56). A virus-induced, RNA-independent phosphatase constitutively present in animal cells catalyzes the dephosphorylation of PKR and eIF-2α (51, 52). The phosphatase has been identified as a type I, manganese-dependent protein phosphatase (52). The ribosome-associated form of PKR appears to be a monomer (22, 53), but the soluble form of PKR-Mus may be a partially phosphorylated homodimer (53). A number of inhibitors of the protein of unknown function (55), which complexes with VAI RNA and with PKR, and is phosphorylated by PKR (54).

PKR is an important component in the antiviral action of interferon (8, 49). Viruses that have deleted from their genome genes that antagonize the action of PKR, for example adenovirus VAI RNA and vaccinia virus K3L, are reported to have an increased sensitivity to wild-type PKR (47). Activators in the PKR activation process include dsRNA at high concentration and three highly structured ssRNA species: adenovirus VAI RNA, Epstein-Barr virus EBER RNA, and HIV TAR RNA. RNA binding proteins that sequester activator RNAs, for example the reovirus s3 protein and HIV TAR RNA, also activate the PKR activation process (48). In addition, 2-amino purine is a potent inhibitor of PKR, although cellular protein kinases in general are not inhibited by the purine analog (48). Some of the most direct evidence for the involvement of PKR in translational repression in mammalian cells emerges from the analysis of various RNA, protein, and purine inhibitors of kinase activity (8, 39, 46, 48).

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binding of GCN2 to ribosomes is not regulated by the availability of amino acids (33). Rather, the catalytic activity of the GCN2 kinase appears to be regulated in response to amino acid levels. The HisRS domain present within the C-terminal half of the GCN2 protein regulates its catalytic activity (15, 17).

GCN2-mediated phosphorylation of eIF-2α occurs in vivo (34, 35), and GCN2 activates the eIF-2α protein kinase (63). Among eIF-2α protein kinases, GCN2 exhibits a unique ability to be activated by amino acid deprivation, and this can be achieved at the transcriptional level; the uncharged tRNALys is postulated to stimulate the GCNB eIF-2α protein kinase (63). Among the GCN2-subtype eIF-2α protein kinases, GCNZ is the best characterized. Although GCNZ appears to be regulated in response to amino acid levels, its activation is not unique to amino acid deprivation. GCNZ (12, 24, 25, 32, 51) and GCNZ-like eIF-2α protein kinases (12, 25, 32) have been isolated from a variety of organisms, including multiple species of fungi, yeast, and mammals (12, 24, 25, 32, 51) such as Homo sapiens (12, 24, 25, 32, 51), GCNZ has three subtypes (GCNZ1, GCNZ2, and GCNZ3), each of which is encoded by a separate gene (12, 24, 25, 32, 51). GCNZ2 is the most abundant of the three mammalian GCNZ subtypes and is expressed in nearly all mammalian tissues (12, 24, 25, 32, 51). GCNZ2 is regulated at the translational level; the uncharged tRNALys is postulated to stimulate the GCNB eIF-2α protein kinase (63). Among the GCN2-subtype eIF-2α protein kinases, GCNZ exhibits a unique ability to be activated by amino acid deprivation, and this can be achieved at the transcriptional level; the uncharged tRNALys is postulated to stimulate the GCNB eIF-2α protein kinase (63). Among the GCN2-subtype eIF-2α protein kinases, GCNZ exhibits a unique ability to be activated by amino acid deprivation, and this can be achieved at the transcriptional level; the uncharged tRNALys is postulated to stimulate the GCNB eIF-2α protein kinase (63). Among the GCN2-subtype eIF-2α protein kinases, GCNZ exhibits a unique ability to be activated by amino acid deprivation, and this can be achieved at the transcriptional level; the uncharged tRNALys is postulated to stimulate the GCNB eIF-2α protein kinase (63).