Cloning and characterization of hIF2, a human homologue of bacterial translation initiation factor 2, and its interaction with HIV-1 matrix

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The cDNA for a human homologue (hIF2) of bacterial (bIF2) and yeast (yIF2) translation initiation factor two (IF2) has been identified during a screen for proteins which interact with HIV-1 matrix. The hIF2 cDNA encodes a 1220-amino-acid protein with a predicted relative molecular mass of 139 kDa, though endogeneous hIF2 migrates anomalously on SDS/PAGE at 180 kDa. hIF2 has an extended N-terminus compared with its homologues, although its central GTP-binding domain and C-terminus are highly conserved, with 58 % sequence identity with yIF2. We have confirmed that hIF2 is required for general translation in human cells by generation of a point mutation in the P-loop of the GTP-binding domain. This mutant protein behaves in a transdominant manner in transient transfections and leads to a significant decrease in the translation of a reporter gene. hIF2 interacts directly with HIV-1 matrix and Gag *in vitro*, and the protein complex can be immunoprecipitated from human cells. This interaction appears to block hIF2 function, since purified matrix protein inhibits translation in a reticulocyte lysate. hIF2 does not correspond to any of the previously characterized translation initiation factors identified in mammals, but its essential role in translation appears to have been conserved from bacteria to humans.

Key words: gene expression, packaging, viral.

INTRODUCTION

A pivotal step in translation initiation is the delivery of the initiator methionine transfer RNA to the small ribosomal subunit. In prokaryotes this is accomplished in part by bacterial translation initiation factor 2 (bIF2), which promotes the binding of fMet-tRNA^{fMet} to the 30 S ribosomal subunit P site [1,2]. bIF2 is a single large polypeptide chain with a non-conserved N-terminal domain which is dispensable for cell viability [3]. It has a central GTP-binding domain and a conserved C-terminal domain that has been implicated in tRNA binding [4]. bIF2 stimulates the association of the 50 S subunit with the 30 S subunit. Upon subunit association, the GTPase activity of bIF2 is activated and it is released from the ribosome (reviewed in [5]).

In eukaryotes the initiator methionine tRNA (tRNA$_i^{Met}$) is delivered by a heterotrimeric complex of proteins called eukaryotic initiation factor two (eIF2). eIF2 forms a ternary complex with Met-tRNA$_i^{Met}$ and GTP which interacts with the 40 S subunit. This complex, together with other initiation factors, associates with mRNA through recognition of the mRNA cap-binding complex and is thought to scan the mRNA for an AUG start codon. eIF5 then triggers hydrolysis of the eIF2-bound GTP, which causes release of the initiation factors from the 40 S subunit and triggers 60 S subunit joining (reviewed in [6]).

Although there are mechanistic similarities in the translation-initiation pathways in prokaryotes and eukaryotes, the factors involved were thought to be unrelated. However, a bIF2 homologue has recently been identified in yeast (yIF2) [7]. yIF2, previously known as Fun12 [8], was shown to be a general translation initiation factor. Purified yIF2 stimulates the formation of methionylpuromycin *in vitro* and can replace eIF2 in this respect. This data indicated that yIF2 might be able to fully substitute for eIF2 in translation initiation. However, extracts from yIF2 knockout yeast strains showed abnormal polysome profiles with an excess of 80 S monosomes. Furthermore, these extracts translated a reporter gene with low efficiency, and addition of the C-terminal half of yIF2 to these extracts restored normal translation. The yIF2 knockout yeast strain exhibited a severe-slow-growth phenotype which was partially suppressed by overexpression of the tRNA$_i^{Met}$ gene, suggesting that yIF2 functions together with eIF2 in the delivery of Met-tRNA$_i^{Met}$ to the ribosome [7].

HIV-1 matrix (p17) is produced as part of the Gag polyprotein precursor (p55), which is proteolytically cleaved at the time of viral budding or shortly afterwards to produce matrix, the structural protein capsid (p24), nucleocapsid (p7), which is involved in packaging of the genomic RNA, p6 and several small polypeptides [9]. Matrix has been implicated in a number of different stages in the viral life cycle, including targeting the Gag precursor to the plasma membrane [10], directing envelope incorporation into virions [11] and the nuclear import of pre-integration complexes [12]. In the present study we report the identification of a human homologue of bacterial IF2, isolated by virtue of its interaction with HIV-1 matrix. This association suggests that matrix may play a role in translational regulation during HIV infection.

EXPERIMENTAL

Yeast two-hybrid screen and cDNA cloning

Yeast transformations and library screens were carried out using a human T-lymphocyte cDNA library in pACT and yeast strain YRG-2 according to the manufacturer's instructions (Clontech), with HIV-1 p17 subcloned into pAS2 as bait. Liquid assays were

Abbreviations used: GST, glutathione S-transferase; eIF2, eukaryotic initiation factor two; IF2, initiation factor two; hIF2, human homologue of bacterial (bIF2) and yeast (yIF2) translation IF2; HA, haemagglutinin; EIAV, equine-infectious-anaemia virus; MLV, murine-leukaemia virus; RACE, rapid amplification of cDNA ends; tRNA$_i^{Met}$, initiator methionine tRNA; ONPG, *o*-nitrophenyl β-D-galactopyranoside; CMV, cytomegalovirus.

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carried out using *o*-nitrophenyl β-D-galactopyranoside (ONPG) as substrate. One unit of enzyme activity is defined as the amount which hydrolyses 1 μmol of ONPG/min. The partial hIF2 cDNA identified in the two-hybrid screen was used to screen a λ ZapExpress HeLa cDNA library [13] for the full-length cDNA by conventional methods. The longest cDNA obtained corresponded to amino acids 371–1220. To obtain the 5′ end of the cDNA, rapid-amplification-of-cDNA-ends (RACE) PCR was used with human heart marathon ready cDNA according to the manufacturer's instructions (Clontech). The full-length cDNA was assembled in pCDNA3 on a *Bam*HI–*Not*I fragment to generate pCDNA3hIF2. pCDNA3HAhIF2, which has an N-terminal addition of the haemagglutinin (HA) epitope, was generated by replacement of a *Bam*HI–*Eco*RI fragment from pCDNA3hIF2 with a *Bam*HI–*Eco*RI PCR fragment obtained using this plasmid as template, generated with the following oligonucleotides: 5′-ggcgggatccgccaccatgtacccctacgacgtgcccgactacgccgggaagaaacagaaaaacaagagc and 5′-ctcccagagtacatttccactttcgg. The point mutation in the GTP-binding domain of hIF2 ($Gly^{643} \rightarrow Arg$) was generated using the Quickchange protocol (Stratagene) using pCDNA3HAhIF2 as template and the following mutagenic oligonucleotides: 5′-ttgggcatgtggacacacggaagacaaaaattct and 5′-agaatttttgtcttccgtgtgtccacatgcccaa. The plasmid containing the point mutation was verified by DNA sequencing and named pCDNA3HAG643RhIF2. DNA sequencing and Northern analysis were carried out as described previously [13], and Northern signals were quantified using a STORM imager (Molecular Dynamics). Sequences were aligned using Pileup in the GCG (University of Wisconsin Genetics Computer Group) suite of programs and shaded using BOXSHADE (http://www.ch.embnet.org/software/BOX-form.html).

Protein and antibody production

Glutathione S-transferase (GST) fusion proteins were expressed in *Escherichia coli* using pGEX2T and purified using GSH–Sepharose. p17 was further purified as described previously [14]. Equine-infectious-anaemia-virus (EIAV) p15 and murine-leukaemia-virus (MLV) p15 were expressed as thioredoxin fusions in pET32a and purified using His bind resin according to the manufacturer's instructions (Novagen). Following cleavage of fusion proteins with enterokinase, the matrix proteins were further purified by ion-exchange chromatography on a Mono S column (Pharmacia). HIV-1 Gag (p17–p7) and GagΔp17 (p24–p7) were purified as histidine-tagged fusion proteins from *E. coli* using a HisTrap kit (Pharmacia). Antibodies were generated to the hIF2 C-terminal domain by inoculation of a rabbit with five 100 μg aliquots of GST–hIF2 (amino acids 921–1220). The antiserum was used at a dilution of 1:5000 in Western blots. Immunofluorescent detection of HAhIF2 was carried out using a Leica confocal scanning microscope with the 12CA5 HA antibody and tetramethylrhodamine isothiocyanate ('TRITC')-labelled anti-mouse secondary antibody. The nuclei and cytoplasms of non-transfected and transfected cells were revealed by using the nucleic acid stain YoPro-1.

Protein interaction assays

GST pull-down assays were carried out as described previously [13], except that reactions were carried out using NP40 lysis buffer [50 mM Tris/HCl (pH 7.5)/100 mM KCl/0.5 % Nonidet P40/0.5 mM EDTA/1 mM PMSF]. In the direct-interaction assays, purified HIV-1 matrix was used in conjunction with GST–hIF2 fusion proteins bound to GSH–Sepharose and detected with anti-p17 monoclonal antibody. Co-immunoprecipitation of matrix with hIF2 used subconfluent 293T cells in 60-mm-diameter dishes co-transfected with 10 μg of pCDNA3H-AhIF2 and 5 μg of a Rev-dependent p17 expression vector pSA64 and 1 μg of the Rev expression vector pCMVRev. Cells were lysed in NP40 lysis buffer and immunoprecipitations were carried out using the HA monoclonal antibody 12CA5 (Boehringer) and Gammabind Plus Sepharose (Pharmacia). Immunoprecipitates were detected with monoclonal antibodies to p17 by ECL® (enhanced chemiluminescence; Amersham).

Translation assays

Transient transfection of subconfluent 293T cells in 60 mm-diameter dishes was performed using calcium phosphate precipitates, and the amount of DNA was kept constant in each transfection by the use of pCDNA3. A 100 ng aliquot of a cytomegalovirus (CMV) promoter-driven β-galactosidase gene (pCMVβ–galactosidase) was used as reporter in transfections and was assayed using a chemiluminescence detection kit (Clontech). Transfections were carried out in triplicate on four separate occasions, and representative data sets are shown. *In vitro* transcription/translation assays were carried out using a T7 polymerase TnT kit (Promega) according to the manufacturer's instructions. Reaction mixtures of volume 10 μl were assembled on ice, and assays were performed at 30 °C for 30 min. All recombinant proteins were dialysed overnight against PBS before addition to the translation reactions. Reactions were stopped by placing the reaction mixtures on ice and immediately assaying them for luciferase activity using luciferin and a Lumac luminometer. The DNA template used was the T7 luciferase control DNA supplied with the kit. For assays where the RNA was recovered to check for integrity, 200 ng of *Sac*I-linearized T7 luciferase control DNA was added to the reaction mixture together with 20 μCi of [^{32}P]UTP (sp. radioactivity 800 Ci/mmol). RNA was recovered by phenol/chloroform extraction and ethanol precipitation and analysed on 1.2 %-agarose gels, followed by analysis using a Molecular Dynamics STORM 860 imager.

RESULTS

Identification of hIF2

In order to identify cellular proteins which interact with the matrix protein from HIV-1, we carried out a yeast two-hybrid screen with matrix as bait using a human T-lymphocyte cDNA library fused to the GAL4 activation domain as prey. A single partial cDNA was identified which shared homology with the C-terminus of yIF2 and bIF2 and was named hIF2. The specificity of the interaction between hIF2 and matrix in the two-hybrid system was determined by measurement of the β-galactosidase activity in total cell extracts from transformants grown in liquid cultures (Table 1). The positive-control plasmids containing p53 and the large T antigen [15] showed a clear interaction, as did matrix and hIF2. However, hIF2 showed no interaction with other retroviral matrix proteins, indicating that it specifically interacts with HIV-1 matrix. The partial cDNA identified in the two-hybrid screen corresponded to amino acids 921–1220 of the full-length hIF2, indicating that matrix binds to the C-terminal domain of hIF2.

To obtain the full-length cDNA for hIF2, a HeLa cDNA library was screened, and a 3.4kb cDNA was identified. However, this cDNA was incomplete, and RACE PCR was used to obtain the authentic 5′ end of the cDNA. The complete cDNA (4.2 kb) was sequenced and found to encode a 1220-amino-acid protein with a central GTP-binding domain (Figure 1). The N-termini of

Table 1 Yeast two-hybrid interactions between hIF2 and retroviral matrix proteins

The C-terminal domain of hIF2 (amino acids 921–1220) fused to the Gal4 activation domain was assayed for interaction with retroviral matrix proteins from HIV-1, EIAV and MLV and the results are displayed as β-galactosidase activities in Miller units [22]. The interaction between p53 and large T antigen was used as a positive control [15]. Values shown represent the average ($\pm$S.E.M.) activities for four independent transformants. Abbreviation: SV40 Tag, simian-virus-40 T-antigen.

Gal4 activation domain fusion	Gal4 DNA-binding domain fusion...	β-Galactosidase activity (Miller units)				
		—	HIV-1–p17	EIAV–p15	MLV–p15	p53
—		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
SV40 Tag		< 0.1	—	—	—	0.69 ± 0.07
hIF2		< 0.1	1.47 ± 0.16	< 0.1	< 0.1	< 0.1

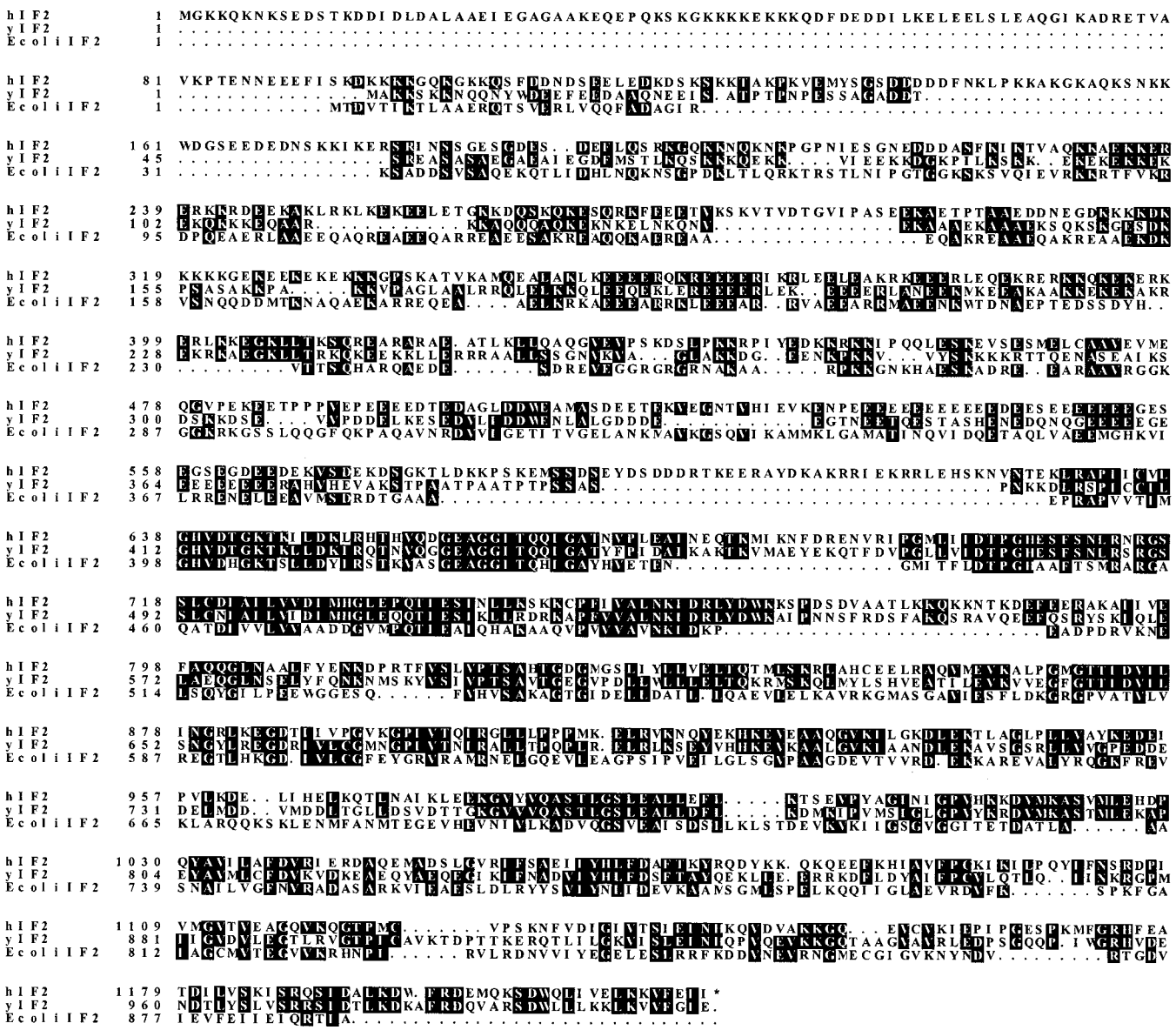

Figure 1 Alignment of hIF2 with IF2 homologues

The GTP-binding domain lies between amino acids 638 and 645 in hIF2. The C-terminal domain of yIF2, which is sufficient for translation functions, starts at amino acid 396 [7]. Identical residues are shown as white letters on black, and amino acid numbers are shown on the left. The accession number for hIF2 in the EMBL, GenBank® and DDBJ Nucleotide Sequence Databases is AJ006776.

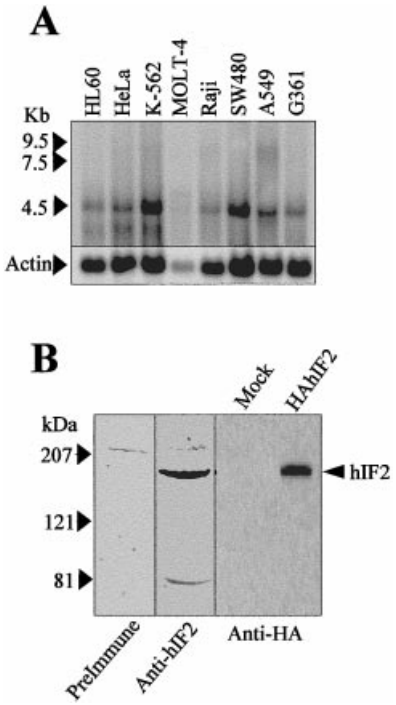

Figure 2 Expression of hIF2 and identification of endogenous hIF2

(**A**) A multiple-cell-line Northern blot was hybridized with a hIF2 cDNA probe (top panel) and rehybridized with actin (bottom panel). (**B**) Endogenous hIF2 was detected by Western blotting using 293T cell extracts with polyclonal anti-hIF2 serum (lane 2), but not with pre-immune serum (lane 1). HA-epitope-tagged hIF2 was detected in 293T cells transiently transfected with 10 μg of pcDNA3HAhIF2 (lane 4), but not with cells transfected with 10 μg of pCDNA3 (lane 3).

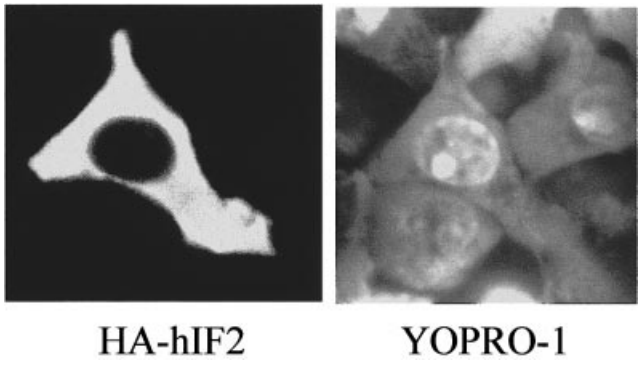

Figure 3 Immunofluorescence localization of hIF2

HA-epitope-tagged hIF2 was detected in 293T cells transfected with 10 μg of pcDNA3HAhIF2 (left panel) and the cytoplasm and nuclei of non-transfected and transfected cells were revealed with the nucleic acid stain YOPRO-1 (right panel).

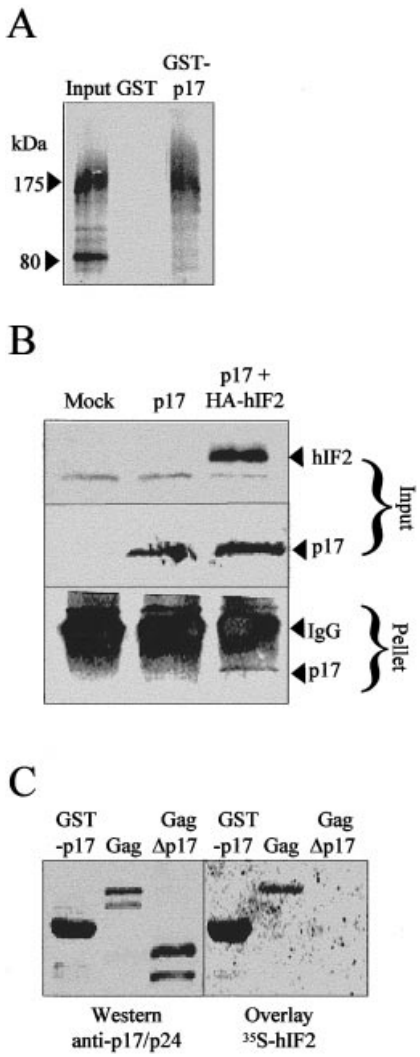

Figure 4 Interaction between hIF2 and HIV-1 matrix (p17)

(**A**) GST or GST–p17 was incubated with [^{35}S]hIF2 and purified using GSH–agarose. Lane 1, radiolabelled hIF2 generated by *in vitro* transcription/translation; lane 2, proteins bound by GST; lane 3, proteins bound by GST–p17. Proteins were detected by phosphorImager analysis following SDS/PAGE separation of bound proteins. (**B**) Co-immunoprecipitation of p17 and hIF2: 293T cells were transfected with pcDNA3 (lane 1, mock), a p17 expression vector (pSA64) (lane 2) or pSA64 and pCDNA3HAhIF2 (lane 3). Immunoprecipitation used the HA monoclonal antibody 12CA5, which was also used to detect HAhIF2 (top panel). A p17 monoclonal antibody was used to detect p17 in the supernatant (middle panel) and pellets (bottom panel). The prominent band in the immunoprecipitates corresponds to the IgG light chain. (**C**) hIF2 binds Gag; purified GST–p17, Gag and GagΔp17 were Western-blotted in duplicate. The left panel was developed using monoclonal antibodies to p17 and p24. The right panel blot was incubated with radiolabelled hIF2 and bound hIF2 was detected by phosphorImager analysis. The higher-mobility bands in the left panel represent degradation products of the recombinant proteins.

IF2 proteins are quite divergent, and hIF2 shows a significantly extended N-terminus compared with its yeast and bacterial homologues. In contrast there is extensive sequence conservation in the C-terminal half of all IF2 family members: for example amino acids 625–1220 of hIF2 have 53 % identity with, and 73 % similarity to, yIF2.

Expression and localization of hIF2

The expression level of hIF2 in a number of cell lines was tested by Northern analysis (Figure 2A), and the major transcript observed was 4.5 kb, consistent with the size of the cloned cDNA (4.2 kb). To identify endogenous hIF2 in cell extracts, a rabbit polyclonal antiserum was raised to the C-terminal domain of hIF2 and used in Western-blot analysis with 293T-cell extracts (Figure 2B). A polypeptide of 180 kDa was recognized by the hIF2

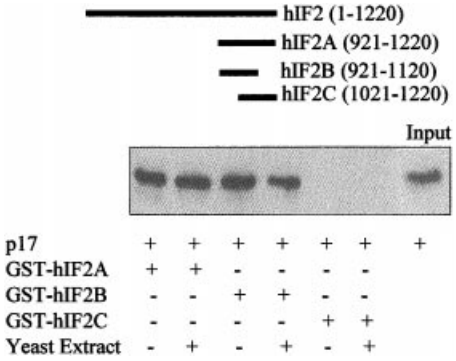

Figure 5 Identification of the p17-binding domain in hIF2

The domains of hIF2 shown (upper panel) were expressed as fusion proteins with GST, incubated with recombinant p17 and purified using GSH–agarose. The proteins added to each reaction are shown in the lower panel. The bound p17 was detected with a p17 monoclonal antibody (middle panel). Some binding reactions were supplemented with a yeast-cell extract as indicated.

antiserum, but not by the preimmune control. This size was higher than that predicted from the amino acid sequence (139 kDa). To confirm that the 180 kDa protein corresponded to hIF2, a HA-epitope-tagged hIF2 was expressed in transient transfections and detected with a HA monoclonal antibody (Figure 2B). HA-hIF2 also migrated at 180 kDa, indicating that the 180 kDa protein recognized by the polyclonal serum was hIF2 and that it migrates anomalously on SDS/PAGE. The subcellular localization of hIF2 was determined in 293T cells transfected with the HA-hIF2 cDNA, and was found to be localized in the cytoplasm, consistent with a role in translation (Figure 3).

Interaction of hIF2 with HIV-1 matrix

To confirm that the full-length hIF2 interacts with HIV-1 matrix *in vitro*, we used a GST pull-down assay with radiolabelled hIF2 (Figure 4A). GST showed no interaction with hIF2 in this assay, but a GST–p17 fusion efficiently bound full-length hIF2. To determine whether this interaction could also be detected *in vivo*, we immunoprecipitated hIF2 from cells transiently transfected with a matrix expression vector (Figure 4B). Matrix was found to co-immunoprecipitate with hIF2 in this experiment, indicating that the two proteins can interact *in vivo*.

The highest intracellular concentrations of HIV-1 matrix in an infected cell occur as part of the Gag polyprotein precursor, whereas the processed matrix entering a cell on primary infection would be present at relatively low concentrations. Consequently we determined whether hIF2 would interact with matrix in the Gag precursor. To do this we utilized recombinant Gag (p17-p7) and Gag deleted for p17 (p24-p7) in a Western overlay assay with radiolabelled hIF2 (Figure 4C). Both GST–p17 and Gag bound hIF2, whereas Gag deleted for p17 did not, indicating that the matrix-binding domain for hIF2 is accessible while it is part of a Gag protein precursor.

In order to demonstrate that the interaction between hIF2 and matrix was direct and not bridged by another factor, we employed a direct GST pull-down assay using purified GST–hIF2 and matrix (Figure 5). The original hIF2 domain identified in the two-hybrid screen (amino acids 921–1220) effectively bound matrix and this interaction was not influenced by the addition of a yeast-cell extract, confirming a direct interaction between the two proteins. The matrix-binding domain in hIF2 was further

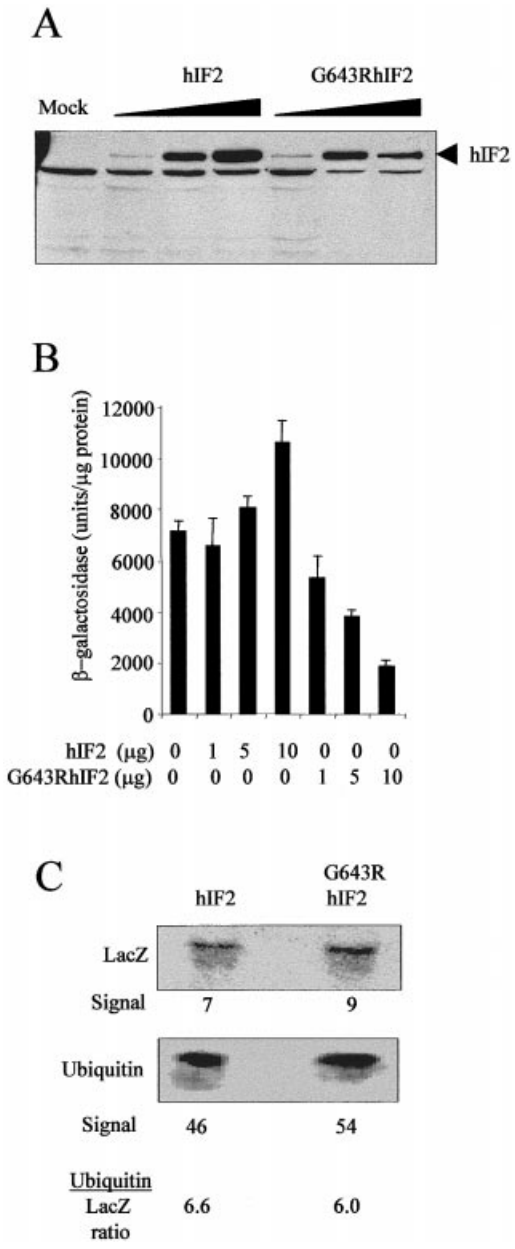

Figure 6 hIF2 is required for general translation

(**A**) G643RhIF2 is expressed in transient transfections: 293T cells were transiently transfected with increasing amounts of pCDNA3HAhIF2 or pCDNA3HAG643RhIF2 and detected by Western blotting with the anti-HA monoclonal antibody 12CA5. Lane 1, mock-transfected cells; lanes 2–4, 1, 5 and 10 μg of pCDNA3HAhIF2 respectively; lanes 5–7, 1, 5 and 10 μg of pCDNA3HAG643RhIF2 respectively. (**B**) G643RhIF2 inhibits translation *in vivo*. 293T cells were transiently co-transfected with a β-galactosidase reporter gene and the indicated amounts (μg) of the HA-tagged hIF2 or the point mutant G643RhIF2, and β-galactosidase activity was measured. The mean value from three independent transfections is shown together with the standard error. (**C**) Northern analysis of 293T cells transiently transfected with the β-galactosidase reporter gene and 5 μg of either wild-type or G643RhIF2 expression vectors. A 10 μg portion of total RNA was loaded per lane and hybridized with a LacZ probe (upper panel); the blot was stripped and rehybridized with a ubiquitin probe (lower panel) and signal strengths shown underneath the blots were quantified using a PhosphorImager.

delineated using truncations of GST–hIF2, and the minimal binding domain was mapped to amino acids 921–1120, which lie at the centre of the C-terminal domain.

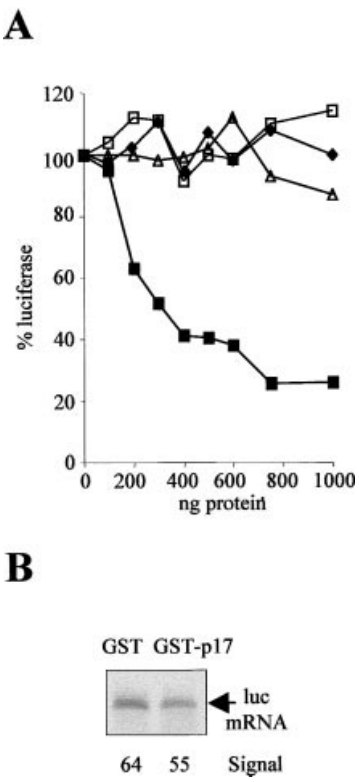

Figure 7 Matrix inhibition of translation *in vitro*

(**A**) Coupled transcription/translation assays were carried out with reticulocyte lysate and a luciferase reporter gene in the presence of increasing amounts of retroviral matrix proteins. Luciferase production was assayed in duplicate reaction mixtures, and mean values are expressed as a percentage with respect to the values seen in the absence of added protein (typically 10 000 arbitrary units). ◆, GST; ■, GST–p17; △, MLV p15; □, EIAV p15. (**B**) *luc* mRNA production in the coupled transcription/translation assay system. Radiolabelled mRNA was generated in the presence of 1 μg of GST (lane 1) or GST–p17 (lane 2), recovered and analysed by agarose-gel electrophoresis. Signal intensity was quantified using a phosphorImager.

hIF2 is required for general translation

The extensive sequence conservation between yIF2 and hIF2 suggested that they may possess conserved functions and that hIF2 may be required for general translation in higher eukaryotes. To determine whether this was the case, a point mutation in the P-loop of the GTP binding domain of hIF2 was generated whereby a glycine residue was changed to arginine ($Gly^{643} \rightarrow Arg$). This mutation was expected to disrupt GTP binding in hIF2. We reasoned that this mutation might result in a transdominant protein which could interact with the translational machinery but not fulfil its normal role owing to its inability to bind GTP, thereby competing with wild-type hIF2. Western-blot analysis was used to confirm that $Gly^{643} \rightarrow Arg$ hIF2 was efficiently expressed in 293T cells (Figure 6A). The effects of $Gly^{643} \rightarrow Arg$ hIF2 expression on cellular translation were monitored by co-transfection of a constitutively expressed β-galactosidase reporter plasmid, and the amount of β-galactosidase produced was determined (Figure 6B). Overexpression of wild-type hIF2 led to a modest (1.5-fold) increase in reporter-gene expression at the highest dose used, suggesting that hIF2 is not limiting for translation in 293T cells. When $Gly^{643} \rightarrow Arg$ hIF2 was expressed, it led to a clear dose-dependent decrease in reporter-gene expression, with a 74% decrease at the highest dose. To confirm that the $Gly^{643} \rightarrow Arg$ hIF2 effects on reporter gene expression were due to altered translation, the level of mRNA produced from the reporter was monitored by Northern analysis and found to be equivalent in the presence of wild-type or $Gly^{643} \rightarrow Arg$ hIF2 (Figure 6C). These data indicate that $Gly^{643} \rightarrow Arg$ hIF2 is a transdominant inhibitor of endogenous hIF2 and blocks translation when expressed in mammalian cells.

HIV matrix can influence translation in a reticulocyte lysate

The ability of HIV-1 matrix to interact with the conserved C-terminal domain of hIF2 suggested that it may influence hIF2 function and affect translation. Therefore we determined whether HIV-1 matrix could alter the translation of a luciferase reporter in a coupled rabbit reticulocyte-lysate transcription/translation system (Figure 7A). The addition of GST had no effect in this assay system, nor did the matrix proteins from EIAV or MLV, consistent with their lack of interaction with hIF2 in the yeast two-hybrid screen. However, GST–p17 showed a dose-dependent inhibition of luciferase production, with a maximal inhibition of approx. 75%. To confirm that GST–p17 was influencing translation and not the stability or production of luciferase mRNA, reactions were performed in the presence of labelled UTP and the mRNA was recovered (Figure 7B). In the presence of 1 μg of GST–p17 the integrity and level of *luc* mRNA were not significantly different from the GST control. These experiments suggest that the interaction of HIV-1 matrix with the rabbit homologue of hIF2 prevents its normal functions and therefore decreases translational efficiency.

DISCUSSION

We set out to identify cellular factors which interact with HIV-1 matrix and play a role in the various functions of this protein. During this screen we identified hIF2, the first reported mammalian member of the IF2 family of proteins. An essential role for hIF2 in translation has been demonstrated by the production of a transdominant mutant that effectively inhibits translation *in vivo*. We did not observe a complete shut down of protein synthesis in these experiments, though this might not be expected for two reasons. Firstly, $Gly^{643} \rightarrow Arg$ hIF2 will autogenously inhibit its own translation once it reaches an inhibitory concentration in the cell, restricting its inhibitory effects on general translation. Secondly, whereas yIF2 was shown to be required for optimal translation initiation in yeast, a yIF2 knockout yeast strain still grows poorly, indicating that translation initiation still occurs, but at a greatly decreased rate. This observation is consistent with the inability of exogenously added HIV-1 matrix to completely inhibit translation in the rabbit-reticulocyte-lysate system.

The extensive sequence homology between hIF2 and yIF2 suggests that hIF2 also plays a role in the translation-initiation process. Surprisingly, despite extensive characterization of the translation-initiation factors in rabbit reticulocyte lysates, no protein has previously been identified with a molecular mass similar to that of hIF2 [6]. However, we have identified a partial rabbit cDNA (GenBank® accession no. AF013270) that shows 99% sequence identity with hIF2, indicating that the protein is highly conserved in mammals. With the exception of eIF2, eIF2A is the only other protein described previously which has the ability to stimulate the formation of methionylpuromycin [16]. However, the reported molecular mass of eIF2A is 65 kDa, consequently yIF2 and hIF2 are unlikely to correspond to this

protein. Clearly, the identification of yIF2 and hIF2 indicates that the traditional scheme for translation initiation in eukaryotes should be modified to include these factors.

The results presented here indicate that HIV-1 matrix associates with the C-terminal domain of hIF2 and has the potential to regulate translation. Interestingly, matrix has previously been shown to interact with another protein involved in translation, HO3, a putative histidyl-tRNA synthetase [17]. However, the role that both these interactions play in the HIV-1 life cycle is not clear at present. One possible function for the hIF2–matrix interaction arises from the observation that the structural proteins for virion production are translated from the same genomic mRNA which is packaged into virions by interaction of the nucleocapsid with the packaging signal [18]. Previous experiments have suggested that a virally infected cell contains separate pools of genomic RNA destined for virion incorporation or translation and that genomic RNA which is being translated is not suitable for packaging [19]. It is possible that HIV-1 generates a pool of ribosome-free mRNA for packaging by restriction of translation initiation, through the matrix inhibition of hIF2, once the intracellular concentrations of Gag have reached a sufficiently high level.

The precise role of yIF2 and hIF2 in translation initiation remains undetermined. Our data have confirmed that hIF2 like yIF2 is required as a general translation factor and is unlikely to be responsible for the translational initiation of a subset of mRNAs. The structure of the bacterial elongation factor EF-G shows remarkable similarity to the aminoacyl-tRNA·EF-Tu·GTP ternary complex and a molecular-mimicry hypothesis has been suggested where during translocation EF-G·GTP binds to the ribosomal A site, occupying the space previously filled by the aminoacyl-tRNA·EF-Tu·GTP ternary complex and, following hydrolysis of GTP to GDP, places the peptidyl-tRNA in the P site [20,21]. Recently it has been suggested that bIF2 shares structural homology with EF-G and, by extension of the molecular-mimicry hypothesis, might bind to the ribosomal A site and direct the association of the fMet-tRNA^{fMet} with the ribosomal P site [2]. It is conceivable that hIF2 serves a similar function in eukaryotes and binds the A site, directing the association of the Met-tRNA$_i^{Met}$ with the P site in conjunction with eIF2. The availability of the hIF2 cDNA should facilitate a more precise dissection of hIF2 function in mammalian translation initiation.

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