general mechanism, as other curved bacteria, such as *Helicobacter pylori*, contain similar proteins.

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Coupled Folding during Translation Initiation

The structure of the eukaryotic initiation factor eIF4E bound to a cognate domain of eIF4G and m^{7}GDP in this issue of *Cell* shows that these factors undergo coupled folding to form a stable complex with high cap binding activity that promotes efficient ribosomal attachment to mRNA during translation initiation.

An early step in the initiation of translation on most eukaryotic mRNAs is the attachment of a 43S preinitiation complex, comprising a 40S ribosomal subunit, initiator tRNA, and eukaryotic initiation factors (eIFs) 1, 1A, 2, and 3 to the 5′ end of mRNA. The complex then scans downstream to the initiation codon. Eukaryotic mRNAs have a 5′ terminal "cap" structure (m^{7}G[5′]ppp[5′]N, where N is any nucleotide) and a 3′ poly(A) tail that synergistically enhance ribosomal recruitment to mRNA.

Ribosomal attachment begins with recognition of the cap by the 25 kDa subunit (eIF4E) of the cap binding complex eIF4F, which also contains eIF4A and eIF4G subunits. eIF4G has binding sites for eIF4E, eIF4A, eIF3, the poly(A) binding protein PABP, and RNA. Mammalian eIF4G is larger than its yeast counterpart, and has additional carboxy-terminal residues that bind Mnk protein kinases and contain a second eIF4A binding site (Figure 1). eIF4A is an ATP-dependent helicase that, as part of eIF4F, unwinds the cap-proximal region of mRNA. Association of eIF4E with eIF4G, which directs the helicase activity of eIF4A to the cap-proximal region of mRNA, is essential for initiation. mRNA unwinding and binding of eIF4G to the eIF3 component of the 43S complex allow ribosomal attachment to mRNA.

The activity of eIF4F is a focal point for the regulation of protein synthesis (Gingras et al., 1999). Mammalian eIF4E binding proteins (4E-BPs) and yeast p20 inhibit cap-dependent initiation by blocking the interaction of eIF4G with eIF4E. Inhibition by 4E-BP is related to its phosphorylation state, so that hyperphosphorylated 4E-BP has the lowest affinity for eIF4E. Mammalian eIF4E also undergoes regulated phosphorylation by the eIF4G-associated Mnk kinases. Phosphorylation of eIF4E is increased in response to stimuli that activate translation. Its significance for eIF4E's function is a topic of current interest (Scheper and Proud, 2002).

The structures of mammalian and yeast eIF4E bound to the cap analogs m^{7}GDP, m^{7}GTP, and m^{7}GpppA are similar (Marcotrigiano et al., 1997; Matsuo et al., 1997). In these structures, eIF4E consists of an unstructured N terminus and an antiparallel β sheet backed by three helices on its convex surface. Binding of cap analogs to a hydrophobic slot on eIF4E's concave surface is stabilized by interactions that include π-π stacking of the m^{7}G base between tryptophan residues, a hydrogen-bonding network that fixes the triphosphate moiety, and additional interactions with the second RNA base. A conserved Tyr-X-X-X-X-Leu-ϕ recognition motif (where ϕ is Leu, Met, or Phe) in eIF4G binds to a phylogenetically invariant site on the convex surface of eIF4E; 4E-BPs contain the same motif and bind to the same site on eIF4E, thereby preventing eIF4E-eIF4G interaction (Marcotrigiano et al., 1999). 4E-BP and eIF4G peptides containing this recognition motif are disordered but assume a helical conformation when they bind to eIF4E, whereas the structure of eIF4E remains unaltered (Marcotrigiano et al., 1999).

Biochemical data suggested that association of eIF4G with eIF4E significantly enhances eIF4E's affinity for the cap (Haghighat and Sonenberg, 1997; Ptushkina et al., 1998). However, interaction of eIF4E with an eIF4G-peptide containing this recognition motif did not enhance eIF4E's cap binding activity, whereas a larger yeast eIF4G fragment containing this motif did (von der Haar et al., 2000). Moreover, mutations outside this motif influenced eIF4G's interaction with eIF4E (Hershey et al., 1999). The observed enhanced affinity of eIF4E for the cap and stabilization of the interaction would significantly increase the efficiency of attachment of 43S complexes to capped mRNAs.

How the interaction between eIF4G and eIF4E led to the enhanced association of eIF4E with the cap remained unknown. Now, Gross et al. (2003) in this issue of *Cell* report the solution structure of yeast eIF4E/cap bound to eIF4G (393–490), which shows that eIF4E's interaction with eIF4G is not limited to the recognition motif but extends over a large (4400 Å^2) interface area. Protein binding results in coupled folding of part of the previously unstructured N terminus of eIF4E (amino acid residues 23–38) to form a "wrist" and a protruding "fist" on the convex surface of eIF4E, and of eIF4G (393–490), which forms a "bracelet" of five helices (with the recognition motif in helix α4) that encircles the "wrist." Kinetic analyses indicated that the initial binding of eIF4E to the

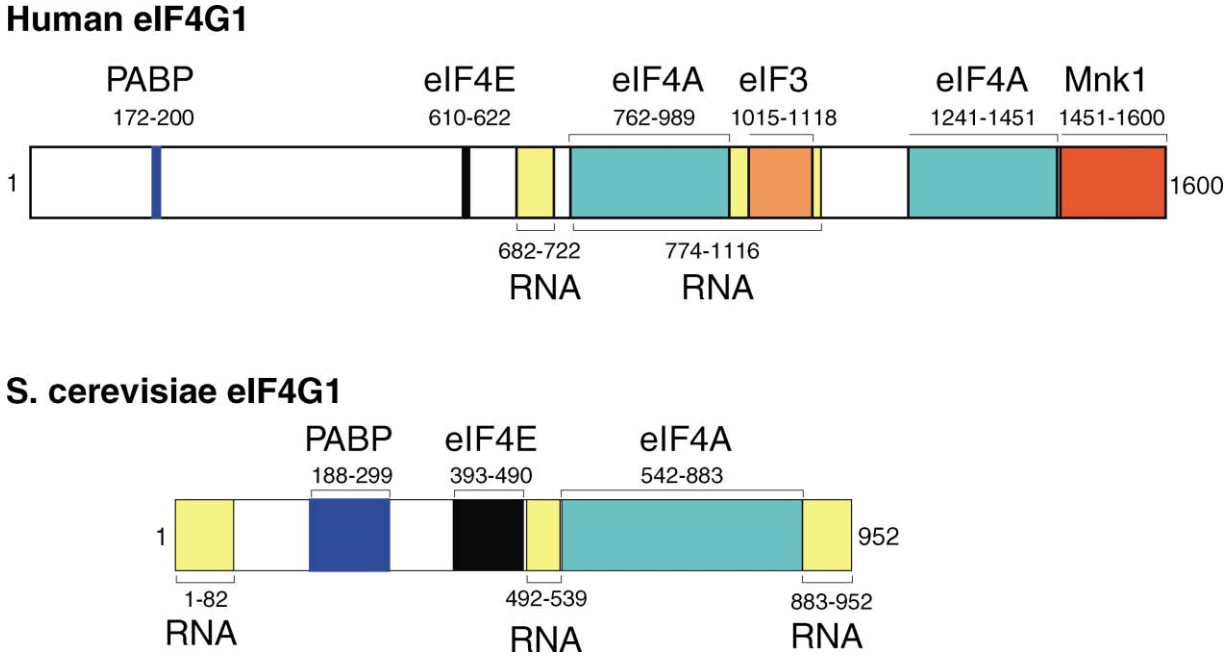

Figure 1. Domain Structures of Human and Yeast eIF4G Proteins

The relative positions and the amino acid numbering of binding domains for RNA and various initiation factors are shown.

recognition motif, which occurs with micromolar affinity, is followed by a second phase during which eIF4E/eIF4G cofolding yields a stable high affinity complex (Kd = 2–5 nM). In addition to promoting tight binding and slow dissociation kinetics for the resulting complex, formation of the interlocking interface between eIF4E and eIF4G allosterically induces conformational changes in the cap binding site of eIF4E that significantly enhance cap binding. In contrast, binding of eIF4E to the eIF4G recognition motif peptide does not change eIF4E's affinity for the cap. The biological importance of the N-terminal region of eIF4E that interacts with eIF4G was apparent from mutational analyses in yeast, which showed that its deletion led to a decrease in the ratio of polysomes to 80S monosomes and to a reduced growth rate.

Phosphorylation of 4E-BP at four sites is an ordered process that likely occurs while it is bound to eIF4E and that promotes its dissociation. Molecular modeling based on the eIF4E/eIF4G (393–490) structure allowed Gross et al. to predict the positions of three of the four phosphoacceptor residues in the eIF4E/4E-BP complex and to suggest the structural basis for regulation of 4E-BPs activity by phosphorylation. Thus, the phosphoacceptor residues point toward acidic patches of eIF4E, and the order of phosphorylation correlates with their accessibility (Gross et al., 2003). These conclusions extend previous suggestions for the structural basis for changes in affinity for eIF4E caused by phosphorylation of 4E-BP (Marcotrigiano et al., 1999).

This exciting structure of the eIF4E/eIF4G complex raises many further questions about the mechanism of eukaryotic translation initiation and its regulation. For example, formation of a tight long-lived eIF4E/eIF4G complex implies the existence of a mechanism for the dissociation of eIF4E from eIF4G so that 4E-BP can gain access to eIF4E. The suggestion by Gross et al. that assembly of stable eIF4E/eIF4G ribonucleoprotein complex at the capped end of mRNA can promote multiple rounds of ribosomal loading onto mRNA raises the question of how mRNA initially binds to the mRNA binding cleft of the 40S subunit and from which nucleotide the anticodon of initiator tRNA in the ribosomal preinitiation complex is able to inspect the mRNA. The work by Gross et al. strongly advances our understanding of the eIF4E/cap/eIF4G interaction but also emphasizes that there is still much to be learned concerning the architecture of eukaryotic initiation complexes and the dynamic structural rearrangements that they undergo during initiation.

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The Secret Life of ACE2 as a Receptor for the SARS Virus

The membrane-associated carboxypeptidase angiotensin-converting enzyme 2 (ACE2) is an essential regulator of heart function. Now, Li at al. identify and characterize an unexpected second function of ACE2 as a partner of the SARS-CoV spike glycoprotein in mediating virus entry and cell fusion.

Many cell surface-associated molecules with diverse sequences, structures, and cellular functions are usurped by viruses for use as their receptors. Receptor identification is important for understanding virus tropism, pathogenicity, and mechanisms of entry, and may help in the development of therapeutics and vaccines, but remains a challenging task. Although the number of identified receptors for human viruses has increased rapidly over the past two decades, the receptors for most of the several hundred known human viruses remain elusive. The receptor for one of the three known human coronaviruses, HCoV-229E, was identified as the human aminopeptidase N (hAPN, CD13) more than a decade ago (Yeager et al., 1992), but the functional receptor for another human coronavirus, HCoV-OC43, remains unknown. However, the overall pace of research on the third human coronavirus, the SARS-CoV, has been amazingly rapid, and, in keeping with this, just months after the virus itself was discovered, the angiotensin-converting enzyme 2 (ACE2) was identified as its receptor (Li et al., 2003).

Li et al. used a straightforward approach—coimmunoprecipitation of the virus attachment glycoprotein (S1) with lysates from cells that are susceptible to virus infection (Vero E6) followed by mass spectrometry analysis of the coimmunoprecipitated proteins. To express the SARS-CoV full-length glycoprotein (S) and S1 in sufficient amounts required for coimmunoprecipitation and functional characterization, they synthesized a codon-optimized gene based on the published sequence of the Urbani isolate (Rota et al., 2003). The observations that ACE2 specifically binds to S1, supports formation of syncytia due to cell fusion mediated by the interaction with S, and mediates infection of cells otherwise inefficient for virus replication that can be inhibited by an anti-ACE2 antibody provide convincing evidence for its receptor function.

In a remarkable series of experiments, Li et al. not only identified the virus receptor, but also demonstrated key characteristics of the membrane fusion process mediated by the ACE2 interaction with S. First, they showed that expression of recombinant ACE2 and S resulted in cell fusion at neutral pH. This finding suggests that low pH and other viral proteins are not required for fusion. The S glycoprotein from another SARS-CoV isolate (Tor2) can also mediate fusion at neutral pH (Xiao et al., 2003), suggesting that the absence of a low pH requirement to trigger fusion is not strain-specific, although more isolates should be tested. It has also been demonstrated that expression of recombinant S from some coronaviruses can lead to syncytia formation at neutral pH (Lai and Cavanagh, 1997). However, it remains possible that low pH is important for uptake of cell-free virus. Second, the S glycoprotein was not cleaved to any measurable degree, but effects of cleavage at the cell surface by proteases on fusion cannot be excluded. Recent biochemical and functional data showed that coronavirus S glycoprotein is a class I fusion protein (Bosch et al., 2003); the lack of cleavage sets apart the SARS-CoV S glycoprotein and spike proteins from other coronaviruses from a prototype class I fusion protein, which is cleaved. Third, the receptor binding domain (RBD) is within the N-terminal fragment containing amino acid (aa) residues 12–672, which Li et al. define as S1. The RBD was recently localized between residues 303 and 537 (Xiao et al., 2003) and is therefore similar to the RBD of the HCoV-229E, which is within a fragment containing residues 407 to 547 (Breslin et al., 2003); whether this reflects any similarity in structure and mechanism of binding of these human coronaviruses is unknown. Finally, Li et al. developed a fusion assay based on syncytia formation that can be used to study mechanisms and to test inhibitors without the need to work with a lethal virus. A pseudovirus-based assay would be a useful complement to control for differences between cell fusion and virus entry.

Preliminary experiments reported by Li et al. also give some initial clues to the molecular mechanism of the ACE2 interaction with S. Two mutations of the ACE2 catalytic site did not affect syncytia formation, indicating that the S binding site on ACE2 is located in a different region and that the enzymatic function of ACE2 is not required for fusion. Although normal cellular function is not usually required for a virus receptor function, further experiments are needed to validate this finding; one possible reason for the lack of effect is related to the long time (48 hr) of syncytia formation (see the supplementary information to Li et al.), which could lead to saturation. The fact that the ACE2-S1 association endured the perils of the coimmunoprecipitation procedure also suggests it may be a high-affinity interaction. The precise affinities of other coronavirus spike-receptor interactions have not been determined (Gallagher and Buchmeier, 2001). However, for most known virus-receptor interactions