X-RAY STRUCTURES OF THE UNIVERSAL TRANSLATION INITIATION FACTOR IF2/EIF5B: CONFORMATIONAL CHANGES ON GDP AND GTP BINDING

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X-ray structures of the universal translation initiation factor IF2/eIF5B have been determined in three states: free enzyme, inactive IF2/eIF5B-GDP, and active IF2/eIF5B-GTP. IF2/eIF5B is a conserved GTPase that facilitates ribosomal subunit joining and Met-tRNAi binding to ribosomes in all three kingdoms of life. The 'chalice-shaped' protein consists of an N-terminal G domain (I), plus and EF-Tu-type β-barrel (II) followed by a novel α/β/α-sandwich (III) connected via a long α-helix to a second EF-Tu-type β-barrel (IV). Structural comparisons reveal a molecular lever, which amplifies a modest conformational change in the Switch 2 region of the G domain induced by Mg^{2+}/GTP binding over a distance of 90Å from the G domain active center to domain IV. IF2/eIF5B interacts with eIF1A through its C-terminal β-barrel domain. This region is critical for growth *in vivo* and translation *in vitro*. Interactions with the ribosome and other components of the translational apparatus are discussed. A model for the mechanism of action of IF2/eIF5B is proposed.

Keywords: TRANSLATION INITIATION GTPASE CONFORMATIONAL CHANGE

THE DISULFIDE BOND ISOMERASE DSBC IS SPECIFICALLY ACTIVATED BY THE IG FOLD DOMAIN OF THE ELECTRON TRANSPORTER DSBD

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Disulfide bonds are important for the structure and function of many proteins, including hormones, antibodies and bacterial toxins. The oxidative folding of proteins with disulfide bonds is catalyzed by thiol oxidoreductases in both bacteria and eukaryotes. The bacterial disulfide bond isomerisation pathway, consisting of DsbC and DsbD, rearranges incorrect disulfide bonds that trap proteins in non-functional conformations. The E. coli protein disulfide bond isomerase DsbC interacts with misfolded proteins and facilitates refolding by rearranging disulfide bonds. DsbC is a V-shaped homodimer with two catalytic domains facing one another across a central uncharged cleft that is the proposed binding site for substrates. The isomerase is specifically activated by the transmembrane electron transporter DsbD. The α domain of the inner membrane protein DsbD has an immunoglobulin fold with two active site cyteines that transport electrons. The intermediate of the electron transport reaction was trapped yielding a covalent DsbC-DsbDα complex. The 2.3Å crystal structure of the complex shows DsbDα binding into the central cleft of dimeric DsbC. The V-shaped DsbC molecule assumes a closed conformation on complex formation allowing both DsbC active sites to interact with DsbDα. These results provide the first insight into the electron transport process associated with oxidative protein folding and explain how DsbC is selectively activated by DsbD using electrons transported from the cytoplasm.

Keywords: PROTEIN DISULFIDE ISOMERASE, OXIDOREDUCTASES, ELECTRON TRANSPORT

EXTRACELLULAR PORTIONS OF EGF RECEPTOR WITH TGFa AND ErbB-2

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Epidermal growth factor receptor (EGFR or erbB1) is the cell surface receptor for EGF, a peptide cytokine that stimulates proliferation in many epithelial tissues. Upon binding extracellular ligand, receptors dimerise into a 2:2 complex, allowing transphosphorylation of the cytoplasmic tyrosine kinase domains. The four members of the EGFR family (erbB1 to 4) can signal as homo- or hetero-dimers in response to a variety of homologous ligands, such as EGF, transforming growth factor a (TGFa), amphiregulin, neuregulin and betacellulin. ErbB2 appears not to bind ligand. Clinically, increased or aberrant signaling via these receptors is characteristic of many cancers. For example, elevated levels of receptor or ligand have been observed in tumors of the brain, head and neck, lung, pancreas and colon. Thus these molecules are good targets for developing new anti-cancer agents. We crystallized ligand-binding fragments of EGFR and erbB2 and determined the structures of the EGFR:TGFa complex and unligated erbB2. Surprisingly, interactions of TGFa with EGFR differ from interactions in other known cell-surface receptor complexes. Ligands usually bind to two or more receptor molecules, bringing them together. Here each ligand is clamped between domains of one receptor molecule and dimerization occurs at a separate receptor:receptor interface. Thus a change in the conformation of the receptor upon ligand binding appears to be an important step in signal transmission into the cell. The structure of erbB-2 shows features of what an unligated receptor might look like and how this receptor can hetero-dimerise without binding ligand.

Keywords: CYTOKINE RECEPTORS COMPLEX CELL SIGNALLING

CRYSTAL STRUCTURE OF A BACTERIAL RNA POLYMERASE HOLOENZYME AT 2.6Å RESOLUTION

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In bacteria, the binding of a single protein, the initiation factor sigma, to a multi-subunit RNA polymerase core enzyme results in the formation of a holoenzyme, the active form of RNA polymerase essential for transcription initiation. We have determined the crystal structure of a bacterial RNA polymerase holoenzyme (the assembly of six protein subunits with a total molecular mass of about 450,000 KDa) from Thermus thermophilus at 2.6Å resolution (R-factor = 22.8%; R-free = 27.4%). The holoenzyme structure provides insight into the structural organization of transcription intermediate complexes, and suggests implications for the mechanism of transcription initiation.

Keywords: RNA POLYMERASE HOLOENZYME, BACTERIA, STRUCTURE