INTRODUCTION

Proteins are the most abundant biological macromolecules that occur in all cells. These are polymers made up of amino acids residues joined by the specific type of covalent bond. Protein molecules, encoded by different genes in DNA genome are the "workhorses" of the cell. The gene expression has two major steps i.e. transcription and translation. During transcription the DNA molecule of a gene act as a template for the complementary base pairing, RNA Polymerase II catalyze the formation of pre mRNA molecule which further processed to a mature mRNA (single stranded copy of a gene) that participate in translation. During translation mRNA acts as a template to synthesize protein. Proteins are derived from the mRNA on the basis of genetic code that relate the DNA sequence to amino acids and then to proteins (Clancy and Brown, 2008).

Protein synthesis occurs with the help of ribosome that acts as a migrating factory that travel along the template (mRNA) engaged in peptide bond synthesis. The machinery which translates the language of mRNA to proteins is composed of four primary components mRNAs, tRNAs, aminoacyl-tRNA synthetase and the ribosome. An mRNA contains a series of codons that interact with the anticodons of aminoacyl-tRNAs so that a corresponding series of amino acids is incorporated into a polypeptide chain (Lewin B., 2004). mRNA consist of a untranslated regions (UTR) at 5’end, this region do not corresponds to particular amino acid and is located between the first nucleotide that is transcribed, and the start codon (AUG) of the coding region. It does not affect the sequence of amino acids in a protein. This region is important in translation because it consists of ribosomal binding sites. 5’UTR region of Eukaryotic mRNA is longer than bacterial mRNA. Length of the 5’UTR region is related to the stability of mRNA and accuracy of translation (Clancy and Brown, 2008).

Protein synthesis is an important part of cellular gene expression (Das and Maitra, 2001). Protein synthesis starts with the following three stages: initiation, elongation and termination. Initiation leads to the formation of the peptide bond between the first two amino acids of the protein. Firstly the ribosome binds to the mRNA, forming an initiation complex that contains the first aminoacyl-tRNA. This is a relatively slow step in protein synthesis and usually determines the rate of mRNA translation. Elongation includes all
the reactions from synthesis of the first peptide bond to addition of the last amino acid. Amino acids are added to the chain one by one; the addition of an amino acid is the most rapid step in protein synthesis. Termination includes the steps that are needed to release the complete polypeptide chain at the same time, the ribosome dissociates from the mRNA (Lewin B., 2004).

Translation initiation is an highly complicated and regulated process that results in positioning tRNA\textsubscript{i}\textsuperscript{Met} (initiator methionyl-tRNA) and the start (AUG) codon in the P-site (peptidyl site) of the ribosome and establish the correct point on an mRNA where translation starts. If translation initiation occurs at the wrong codon it will be potentially a serious problem for cell because it will result in the production of a miscoded protein. Thus, numerous steps have been incorporated to ensure that the correct start site is chosen. Although there is a common basic pathway for translation in all domains of life, the components and their roles in the process differ between bacteria, archaea and eukaryotes (Acker and Lorsch, 2008). Bacteria use three IFs (initiation factors) to hold a ribosome on an mRNA. IF1 binds to the A-site (acceptor site) of the small (30S) ribosomal subunit (Carter et al., 2001) and here it may block the fMet-tRNA\textsubscript{i} (initiator formyl methionyl-tRNA) binding, instead facilitating its binding to the P-site. IF2, a GTPase protein and binds the fMet-tRNA\textsubscript{i} both on and off the ribosome and therefore facilitates the binding of 50S ribosomal subunit. IF3 is an anti-dissociation factor that plays an important role in recycling. Indirectly, it monitors the codon-anticodon interactions between the mRNA and fMet-tRNA\textsubscript{i} in the ribosomal P-site. Bacterial mRNAs contain a Shine-Dalgarno sequence which is present at 5'UTR region is complementary to a short segment of the 3'end of the small ribosomal subunit (16S) rRNA that helps to position the start codon (AUG) in the ribosomal P-site (Laursen et al., 2005).

Translation initiation in archaea follows a hybrid mechanism, archaea utilize some factors that are more closely resemble to those in bacteria as well as in eukaryotes. Archaea contain two initiation factors i.e. aIF1A and aIF5B (aIF, archaeal initiation factor). These factors are related to bacterial IF1 and eukaryotic eIF5B which have overlapping roles in all three domains. Archaea also have orthologues of eIF2, which help in loading the tRNA\textsubscript{i} (initiator tRNA) on to the ribosome (Kyrpides and Woese,
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The tRNA_i in archaea is formylated as in bacteria and like the initiation pathway itself contains a mixture of the sequence elements from both bacterial and eukaryotic tRNA_i (Ramesh and RajBhandary, 2001). However, most archaeal mRNAs are similar in structure to bacterial mRNAs containing Shine-Dalgarno-like sequences and lacking 5’caps. Finally archaea do not appear to encode orthologues of eIF5, a GAP (GTPase-activating protein) for eIF2 or the eIF3 or eIF4F complexes, which are thought to act as scaffolds in eukaryotic translation to organize the initiating ribosome and the highly structured mRNA for the many interactions they must form.

Eukaryotes require a significantly larger number of components at least 12 IFs to accomplish essentially the same goals that bacteria can achieve with just three factors. Two of these components are universally conserved and perform some of the same functions in bacteria, archaea and eukaryotes. eIF1A, the eukaryotic orthologue of IF1 (Kyrtides and Woese, 1998) is thought to bind in a similar spot as it does in bacteria, near the A-site of the small (40S) ribosomal subunit and promote Met-tRNA_i^{Met} binding to the P-site. Likewise, eIF5B is the eukaryotic counterpart of IF2 and interacts with the initiating 80S ribosome in a position similar to binding site of IF2 on the 70S ribosome (Acker and Lorsch, 2008). One fundamental difference between eukaryotic and prokaryotic translation initiation is that the former is quite stringent in only selecting an AUG codon as the start site for translation initiation. For example, none of the nine possible point mutations of the AUG start site at the HIS4 locus in yeast can serve as a signal for translation initiation (Donahue and Cigan, 1988). In contrast, prokaryotic translation initiation at some genes uses alternative codons, such as UUG and GUG (Gualerzi and Pon, 1990).

Translation initiation is the rate-limiting step during polypeptide synthesis and hence an important point of regulation. In eukaryotes there are three different mechanisms of translation initiation, cap dependent, cap independent, reinitiation. The Cap-dependent initiation is the most frequent and best understood. Translation initiation prepares the ribosome to start reading an mRNA to synthesize the corresponding protein. The main steps are to load the methionyl initiator tRNA (Met-tRNA_i^{Met}) into the ribosomal peptidyl (P) site, bind the mRNA, locate the initiation codon and join the small and large ribosomal subunits to form the final initiation complex (Acker et al., 2009).
1.1. Mechanism of Translation initiation in eukaryotes

The process of translation initiation involves the formation of a ribosomal initiation complex where the anticodon of methionyl initiator tRNA (Met-tRNA\textsubscript{i,\text{Met}}) is base-paired with the start codon in the mRNA. In eukaryotes, AUG selection is highly stringent as the start codon and in most cases, the AUG codon closest to the 5′ end of mRNA is used as start codon. Numerous protein factors involved in translation initiation, called eukaryotic initiation factors (eIFs) interact with the 40S ribosomal subunit to stimulate the formation of the translation initiation complex. According to a widely accepted model, eIF3 the large multi subunit factor firstly binds to the free 40S ribosomal subunit and obstruct its association with the 60S ribosomal subunit. The Met-tRNA\textsubscript{i,\text{Met}} interacts with eIF2 (a heterotrimeric factor) which is bound to a molecule of GTP to form a ternary complex is then delivered to the 40S subunit to form 43S preinitiation complex. The resulting 43S preinitiation complex join with an mRNA molecule containing eIF4F bound to 7GpppN cap structure at the 5′ end. The mRNA-bound 48S complex facilitates the recognition of AUG start codon by the anticodon of Met-tRNA\textsubscript{i,\text{Met}}, the process is known as scanning. The correct codon-anticodon pairing lead to the hydrolysis of the GTP bound to eIF2 in a reaction that is stimulated by the GTPase activating protein (GAP) eIF5 and probably modulated by eIF1. Hydrolysis of GTP stimulates the release of eIF2-GDP and other initiation factors from the complex, producing a 40S initiation complex that can join with the 60S subunit. eIF5B (yIF2 in yeast) (Choi et al., 1998) was found to interact with the 60S subunit and stimulate subunit joining in a reaction that consumes a second molecule of GTP (Pestova et al., 2000). The resulting 80S initiation complex with Met-tRNA\textsubscript{i,\text{Met}} bound to the P-site is ready to accept the next incoming aminoacyl-tRNA in the A-site. Because only the GTP-bound form of eIF2 can bind Met-tRNA\textsubscript{i,\text{Met}}, the GDP bound to eIF2 at the end of this process must be replaced by GTP in a reaction dependent on the heteropentameric guanine nucleotide exchange factor (GEF) eIF2B (Asano et al., 2000). One of the essential protein eIF5 plays key role in regulating translation initiation.

1.2. eIF5

Eukaryotic translation initiation factor 5 (eIF5) is a monomeric protein. In mammals it is of about 49 kDa and in yeast (Saccharomyces cerevisiae) it is of 46 kDa (Das et al,
eIF5 acts as a GAP for eIF2 and ubiquitously expressed in yeast, plants, mammals and other eukaryotes. eIF5 binds directly to eIF2β and stimulates GTP hydrolysis only in the eIF2 bound to 40 S ribosomal subunits (Wei et al., 2006).

Genetic and biochemical evidence demonstrates that eIF5 is divided into two domains with distinct functions, the N-terminal domain (eIF5-NTD) that is required for the eIF5-induced GTP hydrolysis and the C-terminal domain (eIF5-CTD) that is responsible for interactions with other eIFs. The eIF5-NTD is highly conserved between eukaryotic species while the eIF5-CTD is only moderately conserved (Wei et al., 2006). Its N-terminal domain functions as a GTPase-activator protein (GAP) for GTP bound to eIF2, while its C-terminal region nucleates the interactions between multiple translation factors, including eIF1, which acts to inhibit GTP hydrolysis or Pi release and the α subunit of eIF2 (Conte et al., 2006). Mutations in eIF2 and its GTPase activating protein, eIF5, that result in an increase in initiation at non-AUG codons in vivo are thought to do so by increasing the rate of irreversible GTP hydrolysis or by increasing the dissociation of eIF2.GTP from the 40S. Met-tRNA_{Met}. mRNA complex (thought to be a pre-requisite for ribosomal subunit joining) prior to GTP hydrolysis (Maag et al., 2006).

Mutations in initiation factors may alter the process of translation. Two important phenotypes, Sui\(^{-}\) (Suppressor of initiation codon mutations) and Gcd\(^{-}\) (General control of derepression) have been used to select mutations in yeast translation initiation factors. Sui\(^{-}\) allows translation initiation from a UUG codon in a mutant HIS4 mRNA, altering the eIFs involved in stringent selection of AUG as the start codon (Asano et al., 1999).

Not much work has been done to identify residues in domain of eIF5 to direct their role in translation. In the present study we would like to dissect eIF5 protein for its role in translation initiation. For this purpose we will mutate eIF5 and analyze the phenotypes using yeast as model system. The information generated from this study will be used in understanding not only translation per se but will also help in studying CK2 mediate cell cycle progression and its related tumorigenesis effects in mammals.