SUMMARY
This thesis reports that both vanadate (+V oxidation state) and different vanadyl species (+IV oxidation state) such as vanadyl D-glucose, vanadyl diascorbate and vanadyl sulfate impair the formation of polysomes and inhibit the initiation of protein synthesis in hemin-supplemented rabbit reticulocyte lysates. Vanadate inhibits protein synthesis more severely than vanadyl species and is consistent with the idea that vanadate is reduced to vanadyl state intracellularly. The inhibition of protein synthesis caused by low concentrations (10-20 μM) of vanadate and vanadyl species is effectively mitigated by reducing agents such as dithiothreitol (DTT), reduced glutathione (GSH) or pyridine dinucleotide (βNADPH). A significant decrease in the protein synthesis inhibition in vanadate-treated lysates by GSH suggests that the mechanism of protein synthesis inhibition by vanadate is different from the action of other oxidants such as heavy metal ions and oxidized glutathione. This suggestion is also consistent with the findings that vanadium compounds do not stimulate phosphorylation of the alpha (α) -subunit of initiation factor 2 (eIF2) or decrease the guanine nucleotide exchange activity of eIF2B, which is required to exchange GDP for GTP in eIF2 GDP binary complex. The reduction of vanadate to vanadyl state and the subsequent complex formation of vanadyl species with the endogenous reducing compounds or with the -SH groups of certain proteins may be the cause for protein synthesis inhibition in lysates.

Further, inorder to determine the defects caused by eIF2α phosphorylation on eIF2B activity and to assess the interaction of eIF2B with phosphorylated and unphosphorylated eIF2α, we have overexpressed here for the first time human eIF2α wild type (wt) and a mutant human eIF2α in which serine 48 was replaced by an alanine
(48A mutant) in the ovarian cells of Spodoptera frugiperda with the help of baculovirus system. Both the wt as well as the 48A mutant of eIF2α were recognized by a monoclonal eIF2α antibody and were phosphorylated by the heme-regulated eIF2α kinase. It was observed that the inhibition in eIF2B activity that occurs due to eIF2a phosphorylation in hemin and poly (IC)-treated reticulocyte lysates was readily decreased in the presence of insect cell extracts overproducing the human 48A eIF2a mutant, but not the wild type. In addition, it has been observed here that the insect cells expressing 48A mutant decreases the formation of [eIF2(aP).eIF2B] complex that occurs between reticulocyte eIF2(αP) and eIF2B in inhibited heme and poly (IC)-treated reticulocyte lysates. These findings support the hypothesis that the 48A mutant of eIF2a reduces the affinity for eIF2B when eIF2α is phosphorylated.

Our studies on insect cell extracts alone indicate that a poly (IC)-induced eIF2α kinase (homologous to human dsl) is absent in insect cell extracts. eIF2B like activity increases in insect cell extracts overexpressing eIF2a 48A mutant. Under non-induced conditions, the over-expressed human eIF2a wild type and 48A mutant do not get phosphorylated in insect cell extracts. These observations in insect cell extracts suggest that a) the eIF2B activity of control insect cell extracts may be inhibited partially due to a low or basal level of eIF2α phosphorylation which can be relieved more efficiently by the expression of 48A mutant or/and b) the dissociation of eIF2.GDP binary complex probably occurs independent of an eIF2B like protein in the presence of eIF2a 48A mutant.