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CONTRIBUTION TO AN UNDERSTANDING OF PROTEIN SYNTHESIS IN RABBIT RETICULOCYTES

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CONTRIBUTION TO AN UNDERSTANDING OF PROTEIN SYNTHESIS IN RABBIT RETICULOCYTES

by

Reena Roy

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TITLE

Contribution to an understanding of protein synthesis in

Rabbit Reticulocytes

BY

Reena Roy

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PREFACE

The format of this dissertation represents a departure from the conventional thesis style. The thesis has been divided into three sections. The first section summarises the present status of protein synthesis initiation. The other two sections represent a complete manuscript by itself. Consequently, the literature review is considerably shorter than in a conventional thesis.

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Dedicated to Ina, Soham and my mother

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ABBREVIATIONS

eIF-2, eukaryotic peptide chain initiation factor 2, forms Met-tRNA$_f$·eIF-2·GTP complex; Co-eIF-2A, a factor stimulating Met-tRNA$_f$ binding to eIF-2; Co-eIF-2B (TDF), ternary complex dissociation factor; Co-eIF-2C, a factor reversing Mg^{2+} inhibition of ternary complex formation by eIF-2; sRF, a factor reversing ·Mg^{2+} inhibition of ternary complex formation by both eIF-2 & eIF-2α(P); ATA, aurintricarboxylic acid; BMV, brome mosaic virus; cpMV, cowpea mosaic virus.

SECTION I

GENERAL INTRODUCTION ON PROTEIN SYNTHESIS INITIATION IN RABBIT RETICULOCYTES

I. INTRODUCTION

There is now convincing evidence that the eukaryotic peptide chain initiation factor 2 (eIF-2) plays a major role in regulation of protein synthesis initiation in mammalian cells. Numerous reports indicate that eIF-2 activity changes under different physiological conditions with accompanying changes in protein synthesis activities in the cells. Austin and Clemens recently reviewed the literature concerning the role of eIF-2 in regulation of protein synthesis in different mammalian cells (1).

eIF-2 forms a ternary complex, Met-tRNA$_f$·eIF-2·GTP as the first step in peptide chain initiation (2-14). Several laboratories have purified eIF-2 to homogeneity (7,8,10-14). The homogeneous eIF-2 is composed of three subunits of approximate molecular weights: α, 32-38,000 daltons; β, 48-52,000 daltons and γ, 50-57,000 daltons. Recently Harbitz and Hauge (13) and Stringer et al. (14) have reported that active eIF-2 preparations from pig liver and rabbit reticulocytes contain only two subunits, presumably α and γ.

Recent work done in different laboratories indicates that several ancillary protein factors such as Co-eIF-2A (15-25), Co-eIF-2B (11,26-28), Co-eIF-2C (29-33), and sRF (34,35) are required for efficient ternary complex formation by eIF-2 and its proper functioning during peptide chain initiation. Co-eIF-2C (29-33) and sRF (34,35) promote ternary complex formation by eIF-2 in the presence of Mg^{2+}. Co-eIF-2A binds to preformed ternary complex and forms a stable quarternary complex, Met-tRNA$_f$·eIF-2·Co-eIF-2A·GTP. The

precise function of Co-eIF-2B in peptide chain initiation is not known. In partial reactions, Co-eIF-2B promotes dissociation of the ternary complexes in the presence of high Mg^{2+} (5 mM) and low temperature (0°C).

An important mechanism of regulation of protein synthesis initiation in mammalian cells involves phosphorylation of the α-subunit (38,000 daltons) of eIF-2 by one or more eIF-2 kinases such as HRI (heme-regulated protein synthesis inhibitor, also called HCR, hemin-controlled repressor (36-38)) (39-42) and dsI (double-stranded RNA activated inhibitor (43-50)) (44-46) leading to loss of interaction of the eIF-2α(P) thus formed with one or more ancillary protein factors (Co-eIF-2B and Co-eIF-2C) (27-33) and concommitant inhibition of overall protein synthesis.

In this article, we will summarize the characteristics of the above ancillary protein factors and also eIF-2 kinases and their roles in the complex regulation of protein synthesis initiation in mammalian cells.

II. Co-eIF-2A

The first indication that a protein factor with no detectable eIF-2 activity can stimulate Met-$tRNA_f$ binding to eIF-2 was provided by the discovery of Co-eIF-2A in reticulocyte ribosomal high salt wash by Dasgupta et al. (15). Reticulocyte Co-eIF-2A activity has since been purified to homogeneity (17,51). The molecular weight of homogeneous Co-eIF-2A preparation is approximately 25,000 daltons (51). Table 1 summarizes some characteristic properties of Co-eIF-2A.

Co-eIF-2A increases both the initial rate and total extent of Met-tRNA$_f$ binding to eIF-2 (15). In the presence of excess Co-eIF-2A, approximately 90 percent of input eIF-2 is bound to Met-tRNA$_f$ (15,17). Also, Co-eIF-2A confers considerable stability to the Met-tRNA$_f$·eIF-2·GTP complexes (17). For example, the ternary complexes formed with homogeneous eIF-2 preparations dissociate extensively in the presence of aurintricarboxylic acid (3 X 10^{-5} M)

TABLE I

Properties of Co-eIF-2A

1.	Mol. wt., 25,000 daltons; single polypeptide (51)
2.	Stimulates (2-3-fold) Met-tRNA$_f$ binding to eIF-2 (15-25).
3.	Binds to preformed Met-tRNA$_f$·eIF-2·GTP (24).
4.	Prevents dissociation of Met-tRNA$_f$·eIF-2·GTP complex by aurintricarboxylic acid, mRNAs and hemin (17,25).
5.	Required for Met-tRNA$_f$ binding to 40S ribosomes (17).
6.	Required for overall protein synthesis in reticulocyte lysates (23).

whereas the same complexes formed in the presence of excess Co-eIf-2A are almost completely resistant to aurintricarboxylic acid under similar experimental conditions (17,25).

Several laboratories have reported the presence of Co-eIF-2A-like activities in different eukaryotic cells such as rabbit reticulocytes (15,17), mouse ascites tumor cells (16), wheat germ (19,20) and

Artemia salina (18,21,22). Osterhout et al., have reported the presence of two factors in wheat germ that stimulate ternary complex formation by eIF-2 (20). Like Co-eIF-2A, both factors form aurintricarboxylic acid-resistant Met-tRNA$_f$·eIF-2 complexes. One of these factors, "Co-eIF-2α", like Co-eIF-2A is a low molecular weight protein (20,000-daltons) while the other factor, "Co-eIF-2β" is presumably a high molecular weight protein complex (20). Wahba and coworkers have reported the isolation of two protein factors, "Co-eIF-2A" (65,000 daltons) and "Co-eIF-2B" (two polypeptides, 105,000 and 120,000 daltons) from Artemia salina (22). Only one of these factors, Co-eIF-2B forms an aurintricarboxylic acid-resistant ternary complex (22). It should be noted, however, that in reticulocytes, two additional factors, Co-eIF-2C (29-33) and sRF (34,35), besides Co-eIF-2A, stimulate ternary complex formation by eIF-2 under different physiological conditions. It is possible that the multiple eIF-2 stimulatory activities in different eukaryotic cells are due to Co-eIF-2C and sRF-like activities rather than Co-eIF-2A.

Requirement of Co-eIF-2A in protein synthesis

In partial reactions, Co-eIF-2A stimulates Met-tRNA$_f$ binding to eIF-2 approximately 2-3 fold. The question arose as to whether Co-eIF-2A is an essential component of protein synthesis or whether the Co-eIF-2A requirement can be overcome by addition of excess eIF-2. To answer these questions, Ghosh-Dastidar et al. prepared antibodies against homogeneous preparations of eIF-2 and Co-eIF-2A