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STUDIES ON THE ROLE OF THE PROTEIN FACTOR RF IN THE REGULATION OF PROTEIN SYNTHESIS INITIATION IN RABBIT RETICULOCYTES

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Studies on the Role of the Protein Factor RF in the Regulation of Protein Synthesis Initiation in Rabbit Reticulocytes

by

Michael J. Grace

A DISSERTATION

Presented to the Faculty of
The Graduate College in the University of Nebraska
in Partial Fulfillment of Requirements
for the Degree of Doctor of Philosophy

Major: Chemistry

Under the Supervision of Professor Naba K. Gupta

Lincoln, Nebraska
April, 1984

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TITLE

Studies on the role of the protein factor RF in the regulation of protein synthesis initiation in rabbit reticulocytes

BY

Michael Judd Grace

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Studies on the Role of the Protein Factor RF in the Regulation of Protein Synthesis Initiation in Rabbit Reticulocytes

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During heme-deficiency in reticulocyte lysates, a latent translational inhibitor, named HRI (heme-regulated inhibitor), which blocks polypeptide chain initiation, is activated. HRI is a protein kinase that specifically phosphorylates the M_r 38,000 subunit of the Met-tRNA$_f$ binding factor eIF-2. A previously reported eIF-2-ancillary protein factor Co-eIF-2 promotes displacement of GDP from the native eIF-2·GDP complex facilitating initiation complex (Met-tRNA$_f$·eIF-2·GTP) formation in the presence of physiological magnesium. Phosphorylated eIF-2 (eIF-2α(P)) inhibits initiation complex formation as Co-eIF-2 does not displace GDP from eIF-2α(P)·GDP.

RF, a high molecular weight lysate supernatant factor, reverses protein synthesis in heme-deficient lysates and also re-

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verses HRI inhibition of initiation complex formation; RF contains Co-eIF-2 activity. RF also contains excess M_r 38,000 subunit of eIF-2 in the free and unphosphorylated form. The excess M_r 38,000 subunit of RF can only be phosphorylated by HRI and ATP in the presence of GDP.

RF which promotes initiation complex formation with eIF-2·[^{3}H]GDP and accompanying GDP displacement can also promote initiation complex formation in the presence of HRI and ATP without accompanying GDP displacement. In the presence of HRI and ATP, the initiation complex formed using RF is active in AUG-codon directed Met-tRNA$_f$·40S formation; Met-tRNA$_f$·40S formation is inhibited by preincubation of RF with GDP. These activities are sensitive to N-ethyl maleimide.

Further fractionation of RF yields a preparation which contains HRI-sensitive Co-eIF-2 activity and does not efficiently reverse protein synthesis initiation in heme-deficient reticulocyte lysates. This preparation does not contain excess M_r 38,000 subunit.

These observations suggest that (1) RF provides unphosphorylated M_r 38,000 subunit to eIF-2α(P)·GDP and restores eIF-2 activity. (2) RF contains Co-eIF-2 activity which has dual functions: (i) stimulation of initiation complex by eIF-2 and (ii) GDP-displacement from eIF-2·GDP during initiation complex formation.

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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 describes the mechanism of inhibition of protein synthesis by eIF-2 kinase and reviews the literature related to this problem. The following two sections represent conventional complete manuscripts consisting of the author's published studies on translational-level control of protein synthesis in eukaryotic cells.

References to Sections II and III:

1. Michael GRACE, Robert O. Ralston, Ambica C. Banerjee, and Naba K. Gupta. Protein synthesis in rabbit recitulocytes: Characteristics of the protein factor RF that reverses inhibition of protein synthesis in heme-deficient reticulocyte lysates. (1982) Proc. Natl. Acad. Sci., U.S.A., **79**, 6517-6521.
2. Michael GRACE, Milan Bagchi, Mir Ahmad, Terry Yeager, Charles Olson, Indrani Chakravarty, Nargis Nasrin, Ambica Banerjee, and Naba K. Gupta. Protein synthesis in rabbit reticulocytes: A study of the mechanism of action of the protein factor RF that reverses protein synthesis inhibition in heme-deficient lysates. (1984) (Submitted for publication).

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Common Abbreviations Used

eIF-2, eukaryotic initiation factor 2, which forms a ternary complex, Met-tRNA$_f$•eIF-2•GTP; Co-eIF-2 (Co-eIF-2A and Co-eIF-2C activities), stimulate ternary complex formation by eIF-2; RF (rescue factor) reverses protein synthesis inhibition in heme-deficient reticulocyte lysates; HRI, heme-regulated translational inhibitor; eIF-2α(P), eIF-2 phosphorylated on the α-subunit (HRI catalyzed); MalNET, N-ethyl maleimide.

NOTE: The designation for the glycerol gradient purified fraction of CM-Sephadex RF has been changed in Section II from the published manuscript in order to correlate with the manuscript presented in Section III. The published paper from the manuscript presented in Section II designates the glycerol gradient purified fraction of CM-Sephadex RF as Fraction VII. In this dissertation, this sample preparation will be referred to as fraction VI B RF; fraction VI B RF in Section III is the same preparation.

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SECTION I

Mechanism of Inhibition of Protein Synthesis by eIF-2 Kinase

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Though evidence for translational control of protein synthesis exists in several eukaryotic systems (1-6), the best studied is the reticulocyte lysate system. It was demonstrated that continued protein synthesis in intact rabbit reticulocytes required the addition of iron to the medium (7,8). It was later shown that this effect could be mimicked in reticulocyte lysates by the addition of hemin (9-14). Reticulocyte lysates contain no mitochondria and therefore cannot synthesize heme.

In erythropoiesis, the reticulocyte is the enucleated precurser to the erythrocyte. In the course of reticulocyte maturation globin synthesis persists longer than heme synthesis (15). Control of protein synthesis by heme in reticulocyte lysates represents an example of the synthesis of an enzyme being controlled by the concentration of its cofactor; regulation is intimately related to cellular differentiation. As a reticulocyte matures into an erythrocyte it extrudes its mitochondria, the source of heme synthesis, resulting in a shut-off of protein synthesis.

The incubation of reticulocyte lysates in the absence of hemin results in an abrupt shut-off of protein synthesis. In heme deficiency, a latent translational inhibitor is rapidly activated (11,16,17) which blocks peptide chain initiation (10,11,14,17,18-21). This inhibitor, called HRI (heme-regulated inhibitor), also called HCR (heme-controlled repressor), has been purified to apparent homogeneity (22,23), and has been shown to be a cAMP-

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independent protein kinase that specifically phosphorylates the M_r 38,000 subunit of eukaryotic initiation factor eIF-2 (22-27). Other eIF-2 kinases have been isolated, both in reticulocytes and in other cell types (5,6,28-34).

These eIF-2 kinases have been shown to inhibit protein synthesis. Regulation of protein synthesis by eIF-2 kinases appears to be a general phenomenon rather than a peculiarity of erythropoiesis.

The initiation factor eIF-2 has been purified to apparent homogeneity by several laboratories (35-37) and shown to consist of three subunits: α, M_r 38,000; β, M_r 52,000; and γ, M_r 54,000. In function, eIF-2 appears to be similar to the prokaryotic initiation factor IF-2 with some differences. eIF-2 forms a ternary initiation complex with the eukaryotic amino acyl initiation tRNA (Met-$tRNA_f$) and GTP, and mediates the transfer of Met-$tRNA_f$ to the 40S ribosomal subunit. IF-2 is smaller (M_r 80,000), and forms a binary initiation complex with the prokaryotic amino acyl initiation tRNA (fMet-$tRNA_f$). No IF-2 kinases have been reported.

Initially, it was proposed that the phosphorylation of eIF-2 by HRI resulted in its subsequent inactivation. The evidence given was: (1) HRI specifically phosphorylates the M_r 38,000 dalton subunit of eIF-2 (24-27); (2) HRI inhibits the initiation of protein synthesis in the lysate, resulting in loss of polysomes and concomitant accumulation of ribosomal subunits (40S and 60S) and 80S monosomes (21,38-40); and (3) the addition of a ribosomal salt

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