THE UNIVERSITY OF MANITOBA

INFLUENCE OF AUTOTROPHIC AND HETEROTROPHIC GROWTH ON RIBOSOME STRUCTURE OF PSEUDOMONAS OXALATICUS OX1.

BY

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"INFLUENCE OF AUTOTROPHIC AND HETEROTROPHIC GROWTH ON RIBOSOME STRUCTURE OF PSEUDOMONAS OXALATICUS OX1"

by

RICHARD TERRENCE MARTIN

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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TO MY WIFE, PARENTS AND FAMILY

ABSTRACT

by RICHARD TERRENCE MARTIN

under the supervision of Dr. P. Y. Maeba

The ribosomes of Ps. oxalaticus OX 1 grown under different cultural conditions were studied. Analysis of ribosomal protein complements of formate - and casamino acid-grown cells by pH 4.5 polyacrylamide disc gel electrophoresis indicated 3 protein band differences could not be shown by two dimensional gels. pH 8.7 disc gel electrophoresis showed 3 additional proteins present in the heterotrophic but not the autotrophic ribosomes. From two dimensional polyacrylamide gel estimates of 53-58 heterotrophic and 50-56 autotrophic ribosomal proteins were obtained. Analysis of the ribosomal subunits also failed to locate and identify the specific alterations involved in adaptation of the ribosome. However, a minimum of twenty-six 50 S and fifteen 30 S heterotrophic and twenty-five 50 S and eighteen 30 S autotrophic ribosomal proteins were identified. Analysis of r RNA indicated that the 23 S

r RNA of intact 70 S ribosomes contained "hidden scission". Dissociation of ribosomes into their respective subunits was accompanied by further breakdown of both 16 S and 23 S r RNA of both autotrophic and heterotrophic ribosomes.

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ABBREVIATIONS

Although growth on formate as sole carbon source can not be classified as true autotrophy, it will be referred to as autotrophy in this thesis. The pathway of incorporation of formate carbon into cell material is analogous to that in autotrophs.

Throughout this thesis, ribosomes and subunits isolated from cells grown autotrophically or heterotrophically will be referred to as "autotrophic ribosomes or subunits" and "heterotrophic ribosomes or subunits", respectively.

The ribosomal proteins are numbered horizontally from left to right according to the vertical distance of migration from the origin of the two dimensional electropherogram with the letters S and L representing proteins of the small, or 30 S, and the large, or 50 S, subunits respectively. This nomenclature has been used to describe ribosomal proteins of E. coli in the "Historical". In this thesis, this terminology has been applied to the Ps. oxalaticus OX1 ribosomal proteins.

A_{260}	-	Absorbance at 260 mμ
A_{260} equivalents of protein	-	Amount of protein extracted from 1 A_{260} unit of ribosome or subunit
ATCC	-	American Type Culture Collection
DNAase	-	Deoxyribonuclease
DTT	-	Dithiothreitol (Cleland's Reagent)
Mg acetate	-	Magnesium acetate
RNAase	-	Ribonuclease
rRNA	-	Ribosomal ribonucleic acid
SDS	-	Sodium dodecyl sulphate
TCA	-	Trichloroacetic acid
TK	-	0.01 M tris-HCl + 0.05 M KCl, pH 7.8
TKM	-	0.01 M tris-HCl + 0.05 M KCl + 0.01 M Mg acetate, pH 7.8
TM	-	0.01 M tris-HCl + 0.01 M Mg acetate, pH 7.8
TM-Urea-DTT	-	0.1 M tris-HCl + 0.01 M Mg acetate + 7.0 M urea + 1.0 mM dithiothreitol, pH 8.0

I INTRODUCTION

The heterogeneity of bacterial ribosomal proteins is well documented (Historical). Measurement of protein content showed that some 30 S proteins exist in less than one copy per ribosome (Kurland et al., 1969; Traut et al., 1969; Voynow and Kurland, 1971; Weber, 1972). On the other hand, some proteins of the 50 S subunit were present in more than 1 copy per ribosome while others were in less than one copy per ribosome (Weber, 1972). These facts suggested that different classes of ribosomes exist in a given cell such that the number of copies of a particular protein reflects an average of the ribosome population (Kurland et al., 1969; Kurland, 1970; Voynow and Kurland, 1971).

Two models based upon this "time-average" hypothesis were suggested, the steady state and static models. In the steady state model the ribosomal protein complement changes as a given ribosome proceeds through initiation, propagation, and termination of protein synthesis. The static model requires distinct classes of ribosomes, each with a specific function and specific protein complement.

A functional basis for heterogeneity of ribosomes was suggested by Deusser (Deusser and Wittmann, 1972; Deusser, 1972) who detected a variation in the protein composition of E. coli ribosomes grown under different conditions, i.e., in rich and minimal medium. Park (1973) demonstrated gross changes in protein composition of ribosomes of Thiobacillus novellus grown under autotrophic and heterotrophic conditions. One explanation for these observations was that under different growth conditions different mRNA's would be produced and translated by specific ribosomes.

However, the T. novellus system was difficult to investigate due to the technical problems encountered in growing the organism. In this thesis, investigation into the influence of growth conditions on ribosomal protein composition was studied employing the organism Pseudomonas oxalaticus OX1 (Khambata and Bhat, 1953). This organism is capable of utilizing sodium formate as its sole source of carbon and energy. Conversion of formate to CO_2 which is fixed via the Calvin cycle closely resembles autotrophic growth (Quayle and Keech, 1959 a, b, c). Growth on formate and adaptation to a more heterotrophic growth, and vice versa, are rapid making this system more suitable for this investigation.

II HISTORICAL

Protein synthesis is a very complex cellular process involving over 100 macromolecules. Such components as the transfer RNA (tRNA) molecules; the amino acid - activating enzymes; various supernatant factors involved in chain initiation, propagation and termination; messenger RNA (mRNA); and the 55-60 components of the ribosome are required for protein synthesis. The ribosome plays a central role in this process serving to bind tRNA and mRNA and interacting with various supernatant factors. It contains peptidyl transferase activity which is required for peptide bond formation and is involved in translocation of mRNA during translation as well as the translocation of nascent peptide chains from the "A" to "P" sites. Obviously, an understanding of the ribosome and its various components is necessary for elucidation of the mechanism of protein synthesis.

The Escherichia coli ribosome is characterized by its sedimentation coefficient of approximately 70 S and molecular weight of 2.6×10^6 daltons of which 65%

is RNA (Tissières et al., 1959). It consists of 2 unique subunits of unequal size that function together to mediate protein synthesis. The small, or 30S, subunit has a molecular weight of 0.8×10^6 daltons (Tissières et al., 1959; Hall and Slayter, 1959; Huxley and Zubay, 1960) consisting of a 16S ribosomal RNA molecule of molecular weight $0.53 - 0.56 \times 10^6$ (Kurland, 1960; Midgely, 1965; Stanley and Bock, 1965) and a protein mass of about $0.23 - 0.28 \times 10^6$ daltons, i.e., 30-33% protein (Craven et al., 1969). The other subunit, the 50 S, is roughly twice the size of the 30 S with a molecular weight of 1.8×10^6 daltons (Tissières et al., 1959; Hall and Slayter, 1959). The 50 S particle consists of a 23 S rRNA with molecular weight $1.0 - 1.12 \times 10^6$ (Kurland, 1960; Midgely, 1965; Stanley and Bock, 1965), a 5S rRNA of molecular weight 0.04×10^6 daltons (Brownlee and Sanger, 1967; Brownlee et al., 1967), and an aggregate protein mass of $0.52 - 0.56 \times 10^6$ daltons (Dzionara et al., 1970; Mora et al., 1970).

The ribosome was pictured as a simple entity, much like a virus, until Waller and Harris (1961) demonstrated its structural complexity. Using starch gel electrophoresis in 6 M urea approximately 20 constituent ribosomal proteins, with an average molecular weight of 25,000 daltons, were identified. In further

work, Waller (1964) resolved over 24 proteins and, as well, ruled out the possibility that this number might be due to artifacts arising through aggregation of proteins. Furthermore the electrophoretic patterns of proteins extracted from 30 S and 50 S subunits were markedly different. Due to his research, the ribosome was pictured as a particle with a heterogeneous population of proteins.

Further work on purification of ribosomal proteins carried out in a number of labs indicated that the complexity of the ribosome was underestimated even by Waller's work. The proteins of the 30 S subunit of E. coli have been fractionated into 21 distinct species (Moore et al., 1968; Fogel and Sypherd, 1968; Craven et al., 1969; Hardy et al., 1969; Kurland et al., 1969; Nomura et al., 1969; Kaltschmidt and Wittmann, 1970 a; Wittmann et al., 1971; Hindennach et al., 1971 a) and that of the 50 S subunit into 34 different proteins (Craven et al., 1969; Dzionara et al., 1970; Kaltschmidt et al., 1970; Kaltschmidt and Wittmann, 1970 b; Mora et al., 1971; Hindennach et al., 1971 b). Aggregation, deamidation, carbamylation, disulphide interactions, nucleotide binding, and partial proteolysis have been ruled out as causes for the large numbers of

observed components (Waller, 1964; Traut, 1966; Möller and Chrambach, 1967).

Using polyacrylamide gel electrophoresis with sodium dodecyl sulphate the molecular weights of the 30 S and 50 S E. coli proteins were determined. The values ranged from 10,900 to 65,000 for the 30 S proteins and from 9,600 to 31,500 for those of the 50 S subunit (Dzionara et al., 1970). These values agreed with molecular weights determined by equilibrium sedimentation (Traut et al., 1969; Craven et al., 1969).

The 21 proteins of the E. coli 30 S subunit have been extensively characterized. All proteins are different with no sequence similarities (Kaltschmidt et al., 1967; Traub et al., 1967; Moore et al., 1968; Fogel and Sypherd, 1968; Craven et al., 1969; Hardy et al., 1969; Hindennach et al., 1971 a). Also, each of the 21 proteins reacts with only its homologous antiserum indicating no sequence similarities exist between the proteins (Kaltschmidt et al., 1967; Traut et al., 1969; Stöffler and Wittmann, 1971).

The 50 S proteins have also been characterized although not as extensively. Although two proteins, L7 and L12, were identical except for the acetylation of the N-terminal serine residue in protein L7 (Möller

et al., 1972; Terhorst et al., 1972) the rest of the proteins were unique (Fogel and Sypherd, 1968; Dzionara et al., 1970; Kaltschmidt et al., 1970 b; Mora et al., 1971; Stöffler and Wittmann, 1971; Hindennach et al., 1971 b).

Comparison of 30 S proteins with those of the 50 S subunit by polyacrylamide gel electrophoresis (Strnad and Sypherd, 1969) and by immunological activity (Stöffler and Wittmann, 1971) indicated that no common structure existed between 30 S and 50 S subunits. However, recently, the proteins S 20 (30 S) and L 26 (50 S) have been shown to share some common sequences.

The first indications that ribosomes are functionally, as well as structurally, heterogeneous came from the work of Staehelin (Staehelin and Meselson, 1965; Raskas and Staehelin, 1967) and Nomura (Hosokawa et al., 1966; Traub et al., 1967). Fractionation of subunit in cesium chloride resulted in the removal of some proteins, the "split proteins", from the subunit and the production of a protein-deficient particle, the "core". These workers produced functional ribosomes by the addition of "split proteins" to the protein-deficient "cores". It was apparent that a number of different proteins were bound to the rRNA to form specific sites with catalytic and binding activities.

Once total reconstitution of the 30 S subunit from 16 S rRNA and 30 S proteins was obtained by Traub and Nomura (1968), investigation into the function of each protein was undertaken. For example, S1 is believed to be responsible for the association between mRNA and the 30 S subunit (van Duin and Kurland, 1971); S 2, S 3 and S 14 are involved in the aminoacyl site of the ribosome (Randall-Hazelbauer and Kurland, 1972); S 21 inhibited the binding of N-formyl methionyl -tRNA (van Duin et al., 1972); L 7 and L 12 are involved in translocation (Highland et al., 1973); and L 11 is believed to be the peptidyltransferase enzyme (Nierhaus and Montejo, 1973). The assignment of specific functions to specific ribosomal proteins confirmed the functional heterogeneity of the ribosomal proteins.

The number-average molecular weight for the 30 S proteins was approximately 20,000 daltons (Waller, 1964; Möller and Chrambach, 1967; Craven et al., 1969; Dzionara et al., 1970). Since the 30 S particle contained only 0.23 - 0.26 x 10^6 daltons of protein, 12 different proteins present as one copy per ribosome would be expected. Consistent with this assumption, 13 of the 30 S proteins were purified and found to exist as one copy per subunit (Moore et al, 1968; Sypherd et al., 1969). However, the identification

of 21 unique 30 S proteins was incompatible with this assumption (Hardy et al., 1969; Kaltschmidt and Wittmann, 1970 a). The total mass of these 21 proteins was approximately 0.44×10^6 daltons or 0.2×10^6 daltons greater than the expected value (Craven et al., 1969; Kurland et al., 1969; Traut et al., 1969; Hardy et al., 1969). Thus there was an excess of protein which could not be accomodated by the 30 S subunit.

Several possible artifacts which could account for this discrepancy have been investigated. Such artifacts as overestimation of molecular weights (Craven et al., 1969), contamination of ribosomes by reversibly bound supernatant proteins or enzymes (Kurland, 1966; Hardy and Kurland, 1966) and partial stripping of proteins during ammonium sulphate purification (Hardy et al., 1969) have been ruled out.

On the basis of stoichiometric analysis, the 30 S proteins were divided into 3 classes: "unit proteins" which were present in 0.8 to 1.0 copies per 30 S particle; "fractional proteins", 0.6 or less copies per subunit; and "marginal proteins" which could be either "unit" or "fractional" (Kurland et al., 1969; Traut et al., 1969; Voynow and Kurland, 1971; Weber, 1972). This data strongly supported the

conclusion that purified ribosomes are structural heterogeneous *in vitro* and that no 30 S protein is present in more than one copy per ribosome (Voynow and Kurland, 1971). The discrepancy between total protein mass per subunit and the aggregate mass of 21 proteins could be compensated for by several proteins being present in less than 1 copy per ribosome.

This structural and functional heterogeneity of 30 S subunits has been interpreted by Kurland (Kurland *et al.*, 1969; Kurland, 1970; Voynow and Kurland, 1971) as a "time-average" of the different states that the ribosome occupied during protein synthesis. Two models have been formulated based upon this interpretation - the steady state and the static models. The steady state model required only one "ribosome" made up of "unit proteins" to form a basic particle. Its protein complement changes during initiation, elongation and termination of protein synthesis. The alteration of functional capacities of the "ribosome" is mediated by the displacement of one set of "fractional proteins" by a second set required for the next function of the ribosome cycle. A basic ribosomal particle consisting of "unit proteins" was also postulated for the static model. In this

case, however, different "fractional proteins" were permanently bound to the basic particle to produce different types of 30 S subunits. Each type was active in all phases of protein synthesis of a particular kind, e.g., translation of a specific mRNA.

When stoichiometric information was compared with 30 S assembly data obtained from reconstitution studies, an interesting correlation arose. "Unit proteins" were found to be proteins that bind to RNA to give particles of higher S value, i.e., those in whose absence particles with S - values of 30 could not be formed. Almost all of the "fractional proteins" were required for restoration of full activity of the ribosome in various functional assay systems (Traub et al., 1967). The absence of fractional proteins did not interfere with the assembly of the 30 S subunit but were required for formation of functional particles (Nomura et al., 1969; Kurland, 1970).

Supporting the steady state hypothesis was the following observation. When 30 S subunits were isolated only a fraction were functionally active. By allowing the proteins of the subunits to exchange with specific 30 S proteins, that fraction of active subunits could be increased (Kurland et al., 1969) suggesting that some proteins were able to exchange

with exogenous proteins. Further support came from experiments in which "fractional proteins" were added to ribosomes participating in specific functions. S 21 addition inhibited (van Duin et al., 1972) whereas proteins S 2, S 3 and S 14 enhanced (Randall-Hazelbauer and Kurland, 1972) binding of N-formyl methionyl -tRNA to ribosomes. It was concluded that S 21 was present in "propagating" ribosomes, i.e., ribosomes involved in peptide chain elongation, and absent in an "initiating" ribosome, i.e., ribosomes involved in the initiation of protein synthesis, whereas S 2, S 3 and S 14 were present in "initiating" ribosomes but not in "propagating" ribosomes. It was suggested that the presence of S 21 prevents ambiquity in translation of the A U G codon during chain propagation by altering the ribosomal configuration of an "initiating" ribosome (van Duin et al., 1972)

Further examination of this concept of different "initiating" and "propagating" ribosomes was undertaken by Bollen et al.,(1972) using matrix-bound mRNA to isolate specific 30 S complexes engaged in initiation. Bollen was able to demonstrate that 3 proteins (S 1, S 2 and one unidentified protein) were drastically decreased in "initiating" ribosomes. Both S 1 and S 2 are