Structural studies of the *Escherichia coli* 70S ribosome

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The dissertation of Barbara-S Schuwirth is approved

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To my family & friends and

to Dirk

“Alles wird gut”

“Everything is going to be alright”

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Abstract: Chapter I

In order to survive environmental stress, organisms down regulate their metabolism and limit protein synthesis by storing inactive ribosomes that are rapidly reactivated when conditions improve. Although the ribosome has been proposed to serve as a physiological sensor for thermal stress, its function under such conditions has not been extensively studied. In *Escherichia coli (E.coli)* Protein Y (PY) is expressed as a consequence of low temperature or extensive cell density. During stationary phase the protein was found mainly in the 70S monosome fraction, to some extent in the 100S ribosomal fraction but was completely absent in polysomes once the cells were transferred to fresh medium (Agafonov, Kolb et al. 2001). *In vitro* experiments showed that Protein Y binds at the subunit interface where it stabilizes the 70S ribosome against dissociation at low magnesium (Maki, Yoshida et al. 2000). Protein Y has been proposed to inhibit translation at the stage of elongation since it inhibits binding of tRNA to the ribosomal A-site (Ye, Serganov et al. 2002). However, this model seems inconsistent with Protein Y being in the monosome fraction *in vivo* (Agafonov, Kolb et al. 2001).

Structural and biochemical data show that PY binds between the A - and P site of the small 30S subunit thereby stabilizing 70S ribosomes. In addition the protein inhibits translational initiation at low but not at normal temperatures. PY is also able to compete with conserved initiation factors that, in bacteria are required for subunit dissociation

Abstract: Chapter II:

The structures of the *E. coli* 70S ribosome has been solved by x-ray crystallography up to 3.5 Å resolution**.** The new crystal form was found to contain two ribosomes per asymmetric unit that have two different conformations that may reflect two stages of the ribosome within the elongation cycle. In addition, the new crystal form has been shown to be suitable for soaking experiments, by using the antibiotic kasugamycin and the Ribosome Recycling Factor (RRF) from *E. coli* and *T. thermophilus*. Difference electron density maps show kasugamycin bound to the ribosome at a resolution of 3.5 Å. Electron density attributed to domain I of RRF can be seen to 8Å in the case of *E. coli* and to 4.5Å in the case of *T. thermophilus*.

The 3.5 Å structure of the empty E. coli ribosome, and the observation that the new crystal form is suitable for soaking experiments can be seen as the starting point for obtaining structures of different ribosome complexes of the translation cycle. These structures should provide a significant advancement towards an understanding of the molecular mechanisms involved in protein synthesis.

Summary

The ribosome is a large ribonucleo-protein complex that is required for protein biosynthesis in all kingdoms of life. In this thesis, functional states of the complete *Escherichia coli* (*E. coli*) 70S ribosome have been studied using a combination of x-ray crystallography and biochemical approaches.

One part of this thesis concerns the regulation of ribosome function under cellular stress, such as cold shock. Together with Antón Vila-Sanjurjo, a former postdoctoral fellow in the laboratory of J.H.D. Cate, I was able to obtain a 11Å crystal structure of the complete 70S *E. coli* ribosome bound to the stress response protein Protein Y (Vila-Sanjurjo, Schuwirth et al. 2004). The structure was complemented by RNA - footprinting and binding data, which enabled us to deduce a mechanism for ribosome inactivation during cold shock. A decrease in temperature had been found to result in the induction of Protein Y expression, which binds and stabilizes the 70S ribosome, thereby serving as a storage protein for the translation machinery. Here, I found that PY binds mainly to the ribosomal P site where it is able to compete with Initiation Factor (IF) 1 and IF3 thereby blocking translation initiation in the cold. Once normal growth conditions recur (37°C), the initiation factors are able to overcome the PY inhibition of protein synthesis allowing ribosomes to follow the pathway into active translation.

Moreover, I obtained a new crystal form of the *E. coli* ribosome that diffracts up to atomic resolution. I was able to solve the structure of the 70S ribosome to 3.5 Å resolution, which allows us for the first time to obtain a structural view of the entire ribosome at near atomic resolution. Two ribosomes

within the asymmetric unit of the crystals were found to have two distinct conformations, which are hypothesized to reflect two functional stages of the ribosome within the elongation cycle.

Additionally, I was able to soak the antibiotic kasugamycin (ksg) as well as the Ribosome Recycling Factor (RRF) into the new crystal form, which resulted in difference maps of up to 3.5 Å resolution for the ksg-bound ribosome and 4.5 Å resolution for the RRF-bound ribosome. These ribosomal complexes help to explain how these factors may influence ribosomal activity.

The 3.5 Å structure of the empty 70S *E. coli* ribosome, and the observation that the new crystal form is suitable for binding experiments involving small chemical ligands as well as larger protein factors, raises our capabilities of obtaining atomic resolution structures of ribosomal complexes in different functional states. Combined, such structures in the future should allow for the recapitulation of the complete translational cycle at the ribosome in great molecular detail.

The structural analyses presented in this thesis provide a basis for attempts in the near future to obtain further high-resolution structures of ribosomal complexes trapped at different stages of the translational cycle, with the ultimate goal of unraveling the molecular mechanisms involved in protein biosynthesis.

Zusammenfassung

Die Funktion des Ribosoms, ein Protein – Ribonucleinsaeure – Komplex, beschraenkt sich auf die Proteinbiosynthese. Im Rahmen dieser Doktorarbeit wurden kristallographische und biochemische Experimente dazu verwendet, um strukturelle Prozesse innerhalb des Ribosoms von *Escherichia coli (E. coli)* aufzudecken.

Einer der Regulationsmechanismen des Ribosoms waehrend kaelte-schock Bedingungen wurde aufgeklaert (Vila-Sanjurjo, Schuwirth et al. 2004). Eine 11 Å Struktur des Ribosoms im Komplex mit Protein Y, einem Kaelteschock-Protein in *E. coli,* konnte zusammen mit Antón Vila-Sanjurjo, einem ehemaligen Mitarbeiter aus dem Labor von J. H. D. Cate, geloest werden. Neben der Struktur halfen biochemische Experimente (RNS - 'foot printing' und Bindungstudien), einen Mechanismus fuer die Inhibition des Ribosoms waehrend Kaelteschock zu postulieren. Ein Herabsinken der Temperatur fuehrt zur Expression von Protein Y, welches and das Ribosom bindet, um es zu stabilisieren. Hier konnte ich zeigen, dass Protein Y den Initiations-Schritt der Translation in der Kaelte inhibiert, indem es mit IF1 und IF3 kompetitiert. Sobald die normalen Temperaturbedingungen wieder hergestellt sind, wird Protein Y durch die Initiationsfaktoren verdraengt, die den Prozess der Translation iniziieren.

Darueber hinaus war es mir moeglich, eine neue Kristallform fuer das *E. coli* 70S Ribosom zu finden, welche Roentgenstrahlen bis zu einer atomaren Aufloesung von 3Å streut. Zum ersten mal wurde die Struktur des gesamten 70S

Ribosoms bis zu einer Aufloesung von 3.5 Å geloest. Diese ermoeglicht es uns einen atomaren Einblick in das Ribosom zu erlangen. Die asymmetrische Einheit der Kristalle beinhaltet zwei 70S Ribosomen-Molekuele, die in zwei verschiedenen Konformationen vorherrschen. Diese koennten das Ribosom in zwei unterschiedlichen Staaten des Translations-Zyklusses widerspiegeln.

Zudem war es mir moeglich, das Antibiotikum kasugamcyin (ksg) sowie den Ribosome Recycling Factor (RRF) in die Kristalle diffundieren zu lassen. Die 70S/ksg Kristalle streuten Roentgenstrahlen bis 3.5 Å, die 70S/RRF Kristalle bis 4.5 Å. Die Bindungsstelle der beiden Liganden im Ribosom konnte so gezeigt werden.

Die 3.5 Å Struktur des 70S Ribosoms sowie die Difussionsversuche mit unterschiedlichen Liganden erhoeht die Wahrscheinlichkeit, dass hoffentlich in naher Zukunft, hochaufloesende Kristallstrukturen von funktionellen Ribosomenkomplexen geloest werden koennen. Dies wuerde unser Wissen ueber den Mechanismus der Proteinbiosynthese in grossem Masse erweitern.

Die hier gezeigten strukturellen Analysen gelten als Voraussetzung fuer weitere hochaufloesende Strukturen des Ribosoms und seiner Ligande, die uns, hoffentlich in naher Zukunft, einen atomaren Einblick in den Mechanismus der Proteinbiosynthese gewaehren.