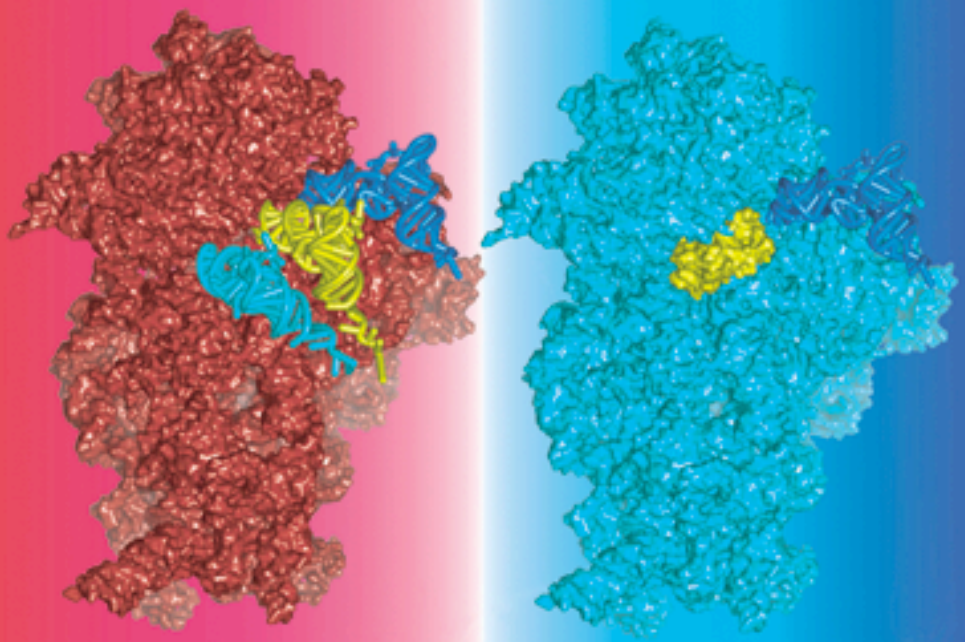
Structural basis for the control of translation initiation during stress



Chapter I

Abstract

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.

Introduction

1. Stationary phase

Under natural conditions bacteria encounter long periods of starvation whereas rapid growth, usually used in laboratory experiments, is the exception. During times of environmental stress cellular metabolism is down-regulated. Morphological and physiological changes (Kolter, Siegele et al. 1993) have been reported in *Escherichia coli* (*E. coli*) cells once they enter the stationary phase resulting in the ability to survive long periods of starvation and stress resistance.

The ribosome is not exempted from changes that occur during stationary phase. The rate of translation and with it the synthesis of ribosomal proteins is known to be down regulated (Zengel and Lindahl 1994) and the active 70S ribosome was found to dimerize in the inactive 100S form (Wada, Yamazaki et al. 1990).

2. Cold-shock versus heat-shock

Apart from extensive cell growth (stationary phase) a temperature change results in a specific stress-response. Depending on the kind of stress, cold-shock or heat-shock a characteristic set of proteins is induced. In *E. coli* most of the proteins that are expressed during cold-shock are involved in translation. One

example for the so called cold-shock proteins is translation initiation factor 2 (IF2) that is responsible for bringing the initiator tRNA (fMet $tRNA^{fMet}$) to the 30S subunit. The expression of IF2 is up-regulated in the cold to ensure translation of cold-shock proteins to occur during stress-conditions. Another example for a cold-shock protein is trigger-factor, a molecular chaperone that binds to the exit tunnel of the ribosome. The RNA-binding protein RbfA, involved in ribosome assembly and CsdA, an ATP-dependent RNA helicase-like protein (Weber and Marahiel 2003) (Gualerzi, Giuliodori et al. 2003) (Inouye and Phadtare 2004) were also found to be expressed during cold-shock. Different from heat shock, where protein folding is the major problem due to denaturation of the proteins at high temperatures, the major problem during cold shock next to the reduction of membrane fluidity is the impaired protein synthesis. In order to function properly in the cold *E. coli* ribosomes require factors, such as RbfA and CsdA. In addition a cold-specific _–factor, like the heat-shock specific transcriptional factor _32 could not be identified so far, suggesting that the cold-shock response is post-transcriptional (Weber and Marahiel 2003).

3. Protein Y

In *E. coli* the stress response protein YfiA, also know as Protein Y (PY) or ribosome associated inhibitor, RaiA (Agafonov, Kolb et al. 2001), is expressed during environmental stress as a consequence of a temperature downshift from 37°C or extensive cell density. PY, with a molecular weight of about 12 kDa,

consists of an N-terminal globular domain of 90 amino acids that adopts an _-_-_-_-_-_ topology and a flexible C-terminal tail (Rak, Kalinin et al. 2002; Ye, Serganov et al. 2002), that was shown to be unimportant for binding of PY to the ribosome (Maki, Yoshida et al. 2000). Most of the conserved regions were found in the N-terminal part, in which the _-helices consist of conserved positively charged residues, which could potentially interact with rRNA once upon binding of PY to the ribosome (**Figure 6A**). The protein was found to be widely conserved within bacteria. In addition homologs have also been identified in two plant species, *Arabidopsis thaliana* and *Spinacia oleracea* (Ye, Serganov et al. 2002). The dsRBD (double-stranded-RNA-binding-domain) from Drosophila Staufen protein (involved in the development of *Drosophila melanogasta* (Ramos, Grunert et al. 2000), known to recognize double-stranded RNA selectively but nonsequence-specificially in a variety of proteins has been postulated as a structural homolog to PY despite limited sequence similarity (**Figure 6B**). Though, after a closer look at the two structures one has to realize that PY's RNA binding domain at the C-terminal tail of the two _-helices does not coincide with the RNA-binding surface of the dsRBDs located in the second and fourth loop region as shown for the Drosophila Staufen protein (Ramos, Grunert et al. 2000) in **Figure 6C**. This is a good example for the fact that proteins, though very similar in their structure, do not necessarily have to interact with their ligands in the same way. Structures are needed to shed light on these issues.

4. *In vivo*

During stationary phase Protein Y was found to be mainly associated with monosomes (70S) and partly binds to the disome (100S, existing of two 70S) fraction. A second stress response protein, YhbH, shows high sequence similarity to PY and is exclusively found in the disome (100S) fraction (Maki, Yoshida et al. 2000). Once the cells were transferred to fresh medium both proteins were found in the 70S fraction, before being removed completely whereas they were absent in the polysome fraction (Maki, Yoshida et al. 2000). The two proteins might function as storage proteins for the ribosome during stress response and may be removed during translational initiation once cell growth has been resumed.

5. In vitro

Protein Y was found to bind at the subunit interface of the 70S thereby stabilizing the ribosome against its dissociation happening at low magnesium concentration. (Agafonov, Kolb et al. 1999). It has been reported that Protein Y inhibits *in vitro* translation elongation by blocking aminoacyl - tRNA binding to the ribosomal A-site. (Agafonov, Kolb et al. 2001). If this was correct PY would be expected to also bind to the polysome fraction. Instead, this model seems inconsistent with Protein Y not being in the polysome fraction *in vivo*.

Ribosomes in all organisms are composed of a small and a large subunit (30S and 50S, respectively, in bacteria) that cycle through stages of association

and dissociation during protein synthesis. During stress PY may function as a translational inhibitor disrupting this cycle through its stabilization of 70S ribosomes (Agafonov, Kolb et al. 1999).

In order to understand the function of protein Y in the adaptation of ribosomes to environmental stress in greater detail, a structural and biochemical approach was chosen. Here, it is shown that PY blocks the peptidyl-tRNA site (P site) and aminoacyl-tRNA site (A site) of the ribosome and inhibits translation initiation during cold shock but not under normal growth conditions.