

Sites of Location of Ribosomes in the Bacterial Cell

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ABSTRACT

Ribosomes, the macromolecular machines that are responsible for mRNA translation into proteins, are described both as complexes freely floating in the cytosol both in eukaryotes and in prokaryotes, and “membrane-bound” to the endoplasmic reticulum in eukaryotes and to the inner face of the cytoplasmic membrane in prokaryotes. For mRNA translation into proteins in bacteria, their cooperation with an elongation factor - EF-Tu in bacteria - is needed. The present communication discusses whether bacterial ribosomes, when active in elongation, might form transient complexes with EF-Tu copies integrated into or bound to filaments that exhibit characteristic features of bacterial cytoskeletons.

Keywords: Ribosome. Cellular location. Eukaryotic cells. Prokaryotic cells. Interaction with EF-Tu. Interaction with cytoskeletal elements?

INTRODUCTION AND HYPOTHESES

For many years, structural and functional organization of ribosomes is a topic that belong to the most important fields of biological research. This is the reason for the overwhelming multitude of available data and the very impressive status of overall knowledge of details (Fischer et al. 2015, Petrov et al. 2015). Nevertheless, one aspect that might need additional research is the fact that possible existing interactions between ribosomes and various cellular components are not yet sufficiently investigated. After all, bacteria lack the rough endoplasmic reticulum (ER), i.e. the assumed attachment site of ribosomes in the cells of higher organisms. In this respect, one should keep in mind that it is not the ribosome proper which is bound to the ER. It is rather the product of ribosome function, the protein that, by the presence of an appropriate amino acid sequence (ER-targeting signal sequence), binds to the ER, penetrates the ER membrane and delivers the protein into the ER lumen. As soon as this has happened, the ribosome is no longer seemingly bound to the ER membrane.

In a typical eubacterium, it is the cytoplasmic membrane that could be a candidate for “binding” of active ribosomes. In fact, the accumulation of ribosomes close to the inner face of the bacterial cytoplasmic membrane is common (s. below). In parallel to the situation in the higher cell, the products of these ribosomes might be proteins finally located within or outside of the cytoplasmic membrane. – As in the cells of higher organisms, individual ribosomes seemingly freely floating in the bacterial cytoplasm are also common. Hence, the question: to

which – if any - of the bacterial cellular substructures – besides the cytoplasmic membrane - are ribosomes bound for functional interaction? Or are they only bound – transiently? - to elongation factor EF-Tu, one of the basic components for ribosomal function, that was shown, in very early investigations, to be freely “floating” in the cytoplasm (Jacobson and Rosenbusch 1976).

EF-Tu was found to be present in a typical bacterial cell in a number of copies much higher than that calculated for the function of “elongation” in the entire population of ribosomes in the cell (Furano 1975). Could this mean an additional function of EF-Tu besides elongation (Beck et al. 1978, Madkour and Mayer 2007, Mayer 2006, 2015)? Or could it indicate two different subpopulations of EF-Tu in the cell? Or could it be just an indication for a comparatively short lifetime of EF-Tu? - One thing should be kept in mind: many years ago (Cremers et al. 1981, Mayer 2006) isolated EF-Tu had been shown to form, in vitro, filaments; later, very similar filaments were observed in partially degraded *Escherichia coli* cells and other bacteria (Mayer 2006). The units of the individual moieties along these filaments had shapes very similar to isolated single EF-Tu proteins (Mayer 2015). Hence, it was discussed that EF-Tu filaments might belong to those filamentous structures in bacteria that form cytoskeletons.

A surprising observation was made – and communicated (Mayer 2006) - a few years ago: in electron microscopic investigations (by application of the negative staining

technique) of *Escherichia coli* cells in a state where the outer membrane was partially removed, groups of particles with the size of ribosomes were found to be arranged, in rows, along helical substructures located close to the inner surface of the cytoplasmic membrane. From earlier and very recent investigations (Defeu Souto et al. 2010, Defeu Souto et al. 2015) it is known that helical protein filaments, containing copies of the protein MreB (a protein of the bacterial cytoskeleton related to actin), do exist in most bacteria.

These filaments are major substructures responsible for the preservation of the cellular shape. Could that mean that the ribosomes are complexed with MreB? Not necessarily: recently it was shown (Defeu Souto et al. 2015) that EF-Tu modulates filament formation of this actin-related protein to affect its localization in *E. coli* and *Bacillus subtilis*. - Prior to that statement, Defeu Souto et al. (2010) had published a finding that may explain the observation described above (groups of particles with the size of ribosomes arranged in helical rows): MreB and EF-Tu colocalize in their experiments. Hence, the particles assumed to be ribosomes do, in fact, not interact with MreB, but rather— due to colocalization of two filament-forming proteins (MreB and, one of them, EF-Tu) - with EF-Tu copies located in the immediate vicinity of the MreB units forming filaments.

This would make sense: during its function as elongation factor, EF-Tu undergoes a close binding with a specific site on the ribosome (Fischer et al. 2015). This binding – needed for proper function of EF-Tu during elongation - is transient, as indirectly demonstrated experimentally by application of the antibacterial agent kirromycin. This agent is known to specifically block the release of EF-Tu from the ribosome after elongation, thereby inhibiting further rounds of elongation of translation (Defeu Souto et al. 2010), with fatal consequence to the bacterial cell.

Here, an earlier observation published several years ago should be mentioned: immune labeling of cells of *Mycoplasma pneumoniae* (a bacterium lacking MreB) (Hegermann et al. 2002) with EF-Tu-specific antibodies revealed an intense labeling close to the inner face of the cytoplasmic membrane of this wall-less bacterium. Labelling of EF-Tu copies distributed within the general cytoplasm of the cells was also observed, but less intense. After removal of the cytoplasmic membrane, the cells maintained their elongated shape; a cell-wide network of filaments all over the outermost layer of the “naked” bacterium (devoid of its cytoplasmic membrane) with defined meshes became visible, and also indications for the existence of filaments crossing the cytoplasm could be obtained by electron microscopy.

This observation supports the view that it is the cell-wide network which stabilizes cell shape (i.e., the network has the function of a cytoskeleton). - Stalks extending from this net structure outward in a direction to the level where the cytoplasmic membrane had been placed (but was now removed) allow the conclusion that, by these stalks, the cytoplasmic membrane had been placed and hold in place prior to its removal. This would mean that the basis of these stalks is located in a net-like delicate layer very close to the inner face of the cytoplasmic membrane. As reported in the results of immune labeling with anti-EF-Tu antibody – dense label close to the inner face of the cytoplasmic membrane – it could be postulated that the cell-wide network forms a true cytoskeleton (s. above) containing a high number of copies of EF-Tu (without the presence of MreB, s. above !). It fits into this image that also the ribosomes, as observed by electron microscopy of ultrathin sections, were preferentially located close to the cell periphery (s. above).

These hypotheses derived from experimental data indicate the need for a number of additional experiments; just to name two relatively simple ones: labeling experiments of “naked” *Mycoplasma pneumoniae* cells (s. above) with anti-EF-Tu antibody. A further example would be experiments performed with *in vitro*-EF-Tu filaments to elucidate binding of ribosomes to these filaments.

This short communication and the listed references demonstrate that our very early suggestion – bacteria possess cytoskeletons - (Mayer et al. 1998) did point in the right direction. In addition, they support the view that a given protein does not necessarily have only one function.

REMARK

Because I retired many years ago from active experimental work, I cannot perform further experiments anymore. Your comments?

ABBREVIATIONS

E. coli – *Escherichia coli*; EF-Tu – Elongation Factor Tu; ER- Endoplasmic Reticulum

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