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Membrane heterogeneity created by transertion is a global regulator in bacteria

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The bacterial membrane is characterized by a heterogeneous distribution of lipids and proteins and of higher level structures termed hyperstructures. The causes of this heterogeneity include lipid–lipid, protein–protein and protein–lipid interactions. The coupling of transcription, translation and insertion of nascent proteins into membrane, transertion, creates large membrane domains that are proposed to be important in the regulation and execution of the cell cycle and in other functions. In describing membrane heterogeneity, we suggest here that transertion is a global regulator coupling metabolism to the cell cycle.

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Introduction

During the past fifteen years, the tremendous progress in protein labeling in live cells and in powerful microscopy techniques has transformed the early picture of the bacterial cell as a simple vessel with well-mixed reagents into the modern vision of a sophisticated machine with compartmentalized components and functions. In particular, evidence has accumulated for the heterogeneous distribution in different species of both the lipid and the protein components of the cytoplasmic membrane. This heterogeneous distribution has led to the idea that membrane domains are involved in both the spatial and temporal organization of bacterial cells. Many important processes in bacteria, from chemotaxis and protein secretion to wall synthesis and cell cycle regulation, are thought to be associated directly with the domain structure of the membrane. Hence, elucidating the origin and structure of these domains is essential to understanding bacteria physiology. Such elucidation must take into account the high degree of structure of both the membrane and the cytoplasm. Here, we review evidence consistent with the coupled transcription–translation–insertion of proteins into membranes (transertion) acting as a global regulator by coupling metabolism with the cell cycle events of chromosome replication and cell division.

Evidence for membrane heterogeneity

Lipid heterogeneity

The idea of a homogeneous distribution of membrane lipids as proposed in the fluid mosaic model [1] has had to be modified in the light of evidence that the distribution of lipids is actually heterogeneous and that this heterogeneity may be important in providing the specific lipid environments needed for the activity of many membrane proteins (for references see [2]). Heterogeneous distributions of lipids have been observed using a variety of techniques in bacteria as different as *Micrococcus luteus* [3], *Mycobacterium* [4], *Bacillus subtilis* [5,6] and *Escherichia coli* [7]. The coexistence of membrane domains with distinct order and polarity in *E. coli* was shown using the membrane probe laurdan [8] and using pyrene-labeled phospholipids [9]. Domains enriched in cardiolipin at the cell poles and near the division sites were shown using a cardiolipin-specific fluorescent dye [10] (for a review see [11]); consistent with this, *E. coli* minicells, which can be thought of as isolated poles or septa, were shown using mass spectrometry to be enriched in cardiolipin [12].

Protein heterogeneity

The protein content of the cytoplasmic membrane of *E. coli* is quite varied, with over 100 species distinguishable on two-dimensional gels [13]. Although the membrane proteins constitute only a small fraction of total cellular content, the bacterial cytoplasmic membrane is one of the most protein-loaded membranes known, with roughly up to 100 phospholipid molecules per protein in a membrane bilayer. It contains numerous transport systems for small solutes, protein translocation machineries, energy generation and transformation systems, peptidoglycan synthesis complexes, chemosensory elements, complexes for phospholipid metabolism, the divisome and the flagella. Many of these proteins are distributed heterogeneously (for a comprehensive review see [14••]). They include integral membrane proteins such as SecE, SecG in *E. coli* and SecA, SecY in *B. subtilis*, Tar, LacY, AtpA, OM proteins and the LPS itself, and peripheral membrane proteins such as FtsA, FtsZ, RNaseE, MreB and MurG (for a longer list and references see [2] and Table S1 in Supplementary material). The heterogeneous distribution of proteins extends to the group of proteins

in the membrane that catalyze peptidoglycan synthesis/degradation [15]; it also extends to phospholipid synthases, the majority of which in *B. subtilis* are located in the septal membranes, consistent with the enrichment of septal membranes in cardiolipin and phosphatidylethanolamine [5]. As in eukaryotic cells, homologs of Flotillin-1, a lipid raft-specific protein, are present in distinct microdomains in *B. subtilis* and *S. aureus* [16]; these microdomains are involved in signal transduction, protein secretion, and biofilm formation. Such involvement in a wide range of processes is consistent with the domain organization of the membrane being important for various types of cells.

One of the most thrilling features of heterogeneity of many membrane and peripheral proteins is that their distribution often resembles a helix with its axis parallel to the long axis of the rod-shaped cell. There is no systematic study of the spatial parameters of such helices and the question of co-localization of different proteins to the same pattern is limited by optical resolution and by the ability to express two or more labeled proteins without interfering with cellular functions. The helical structuring was first ascribed to the polymerization characteristics of the proteins, one of which, the actin-like MreB became the leading candidate for the role of spatial organizer of the cell [17,18]. Subsequent studies, however, have revealed that MreB complexes move independently as patches along a helical path [19••,20••]. MreB forms a proteolipid domain with fluid phospholipids, disruption of which affects the activity of LacY and the F_1F_0 ATP synthase [H. Strahl and L. Hamoen, personal communication]. Notably, the continuous nature of the MreB polymer may be a protein-tagging artifact [21•,22•].

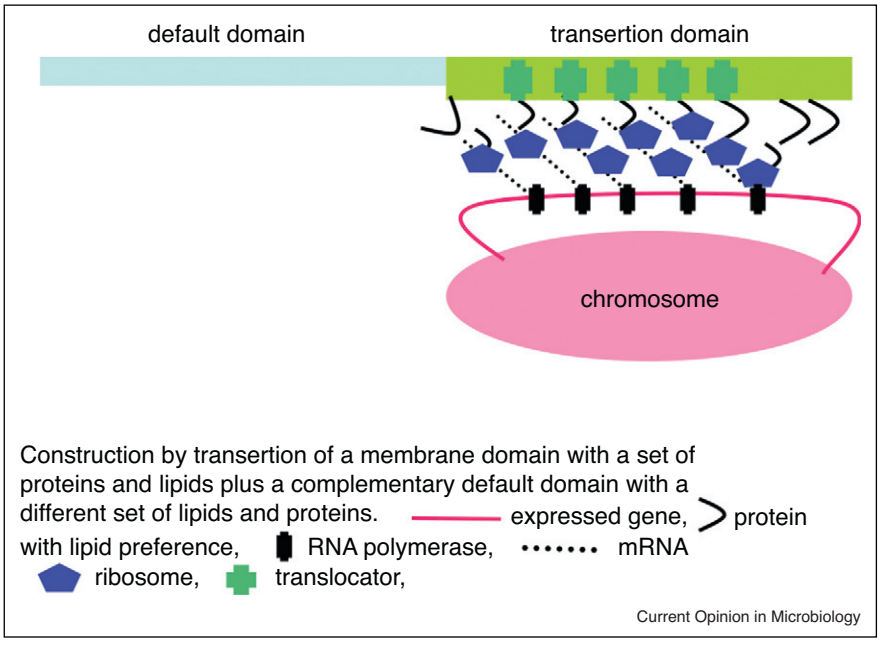
Generation of membrane heterogeneity

Initiation of organization by transertion

Membrane heterogeneity in bacteria as revealed by microscopy involves relatively large structures. In general, both lipid–lipid and lipid–protein interactions are suspected to induce the formation of microdomains, which comprise at most some tens of molecules of a specific lipid. The major driving forces for microdomain formation are: (i) hydrophobic mismatch, (ii) polar interactions with lipid head groups, and (iii) specific protein–protein interactions [23]. What is the mechanism of domain formation in bacterial membrane? Is it spontaneous or is there a fundamental mechanism responsible for organizing heterogeneity? In view of the fact that none of the bacterial cytoskeletal proteins forms a persistent structure, we consider here an alternative mechanism to proteins dedicated to spatial organization. This alternative is a consequence of the functioning of the essential cellular processes of transcription, translation and insertion into membrane of proteins.

It has been proposed that DNA attachment to the inner membrane is brought about by the 'transertion' process—coupled transcription/translation and insertion of integral proteins into and through the membrane (Figure 1). High-level expression of a membrane protein gene

Figure 1



Transertion.