Biochemistry & Analytical Biochemistry
ISSN: 2161-1009

Norris et al., Biochem Anal Biochem 2015, 4:4
DOI: 10.4172/2161-1009.1000234

Commentary Open Access

Extending the Transertion Hypothesis

Vic Norris[1], Eugenia Mileykovskaya[2] and Kouji Matsumoto[3]*

[1]Laboratory of Microbiology Signals and Microenvironment EA 4312, Department of Science, University of Rouen, Mont-Saint-Aignan, France
[2]Department of Biochemistry and Molecular Biology, University of Texas Medical School at Houston, Houston, TX, USA
[3]Department of Biochemistry and Molecular Biology, Graduate School of Science and Engineering, Saitama University,Saitama, Japan

Abstract

The coupling of transcription, translation and the insertion of nascent peptides into membrane (or their association with membrane), termed transertion is increasingly been seen as a major process in the structuring of bacteria. This commentary focuses on current and new ideas about the roles of transertion in processes as varied as lipid metabolism, RNA degradation, osmoregulation and the structuring of nucleoid and membrane. We also discuss the problems that transertion may pose to cells.

Introduction

For many years, transcription and translation were considered as separate processes that occurred separately. This was despite strong experimental [1] and theoretical [2] evidence that the processes were physically coupled. Such coupling was later considered to have emergent properties. In the case of the coupled transcription, translation and insert of proteins into and through membrane, *alias transertion*, these coupled processes have long been proposed to structure membranes and nucleoid [3,4], to direct chromosome replication, chromosome segregation and cell division [5-7], and to underpin differentiation [8]. It has now become apparent that transertion is a major process in its own right [9]. Extensive proteo-lipid domains have been found in the membranes of both *Escherichia coli* and *Bacillus subtilis* [10-17] and transertion has been implicated in such structuring [18,19]. Moreover, the extent of structuring of the nucleoid by transertion has now been shown [20] and the relationship between ribosome location and transertion has been elucidated [21,22], confirming transertion's role as a global regulator [9,19]. In what follows, we review ideas 'under construction' about the relationship of transertion to a variety of bacterial structures and processes including the nucleoid and membrane, RNA degradation, osmoregulation, lipid metabolism, the regulation of the cell cycle and the origins of life.

Causes of Transertion

Peripheral membrane proteins may be important in transertion

In the first versions of the transertion hypothesis, the structuring of the membrane and the tethering of genes was limited to those genes encoding proteins that were either inserted into membranes (such as integral membrane proteins) or secreted/exported through membranes. The latter class has not received much attention although Braun's lipoprotein, for example, might well have a role in transertion if the lipid modification at the NH_2-terminus of the lipoprotein were to occur before the synthesis of the rest of the protein. Importantly, another class of genes – those encoding peripheral membrane proteins – might also structure the membrane and tether genes, thereby contributing to the level of transertion in the cell; these genes include those encoding Noc in *B. subtilis*, which has an N-terminal, membrane-binding, amphipathic helix [23], and, in *E. coli*, SeqA [24], which forms part of a membrane-binding complex [25] and DnaA, where the surface of domain III is responsible for membrane binding [26]. These genes also include *mreB*, which encodes the 'actin-like' MreB; this protein has an N-terminal membrane-binding sequence, which takes the form of an amphipathic helix in *E. coli* and a membrane insertion loop in *Thermotoga maritima* [27].

RNA degradation may play a part in the dynamics of transertion hyperstructures

The RNA degradosome is a hyperstructure belonging to the *functioning-dependent* class [28] because it depends for its existence on an activity that is due to the presence of its substrate (here, RNA [29]). RNase E, which forms the scaffold of the RNA degradosome, diffuses over the entire inner membrane of *E. coli* to generate short-lived hyperstructures [29]. This means that degradosomes are separated from the sites of transcription and this should favour the translation of nascent transcripts by polyribosomes, that is, favour transcription and translation being coupled rather than separated [29].

Transertion and Osmolality

It is becoming apparent that transertion is implicated in sensing various conditions. An increase in osmolality leads to transcriptional activation of the *cls* gene in *E. coli* [30], consistent with an increase in CL levels being a general physiological response to osmotic stress that protects microorganisms from lysis [31]. The polar location of CL is correlated with the polar location of the osmosensory transporter ProP, which actively transports osmo-protectants into the cell [30,32].

Transertion and Lipid Metabolism

The coordination of lipid metabolism with environment conditions may be helped by the assembly of a hyperstructure containing membrane components. Such hyperstructures might be based in part on the transertion of enzymes responsible for phospholipid and LPS synthesis. Significantly, acyl carrier protein (ACP), part of which in *E. coli* is located in the membrane, interacts with enzymes involved in many biosynthetic pathways, including those involved in phospholipid and LPS synthesis in or on the membrane as well as those involved in the synthesis of fatty acid in the cytoplasm. In particular, ACP interacts with PlsB, a *sn*-glycerol-3-phosphate acyltransferase, and PssA, a PS synthase, as well as YbgC, an Acyl-CoA thioesterase involved in fatty acid synthesis [33]. In *E. coli*, PssA, which has a preference for acidic

***Corresponding author:** Kouji Matsumoto, Department of Biochemistry and Molecular Biology, Graduate School of Science and Engineering, Saitama University, Saitama, Japan, Tel: +81-48-858-3345; Email: koumatsu@mail.saitama-u.ac.jp

Received: December 28, 2015; **Accepted:** December 29, 2015; **Published** December 31, 2015

Citation: Norris V, Mileykovskaya E, Matsumoto K (2015) Extending the Transertion Hypothesis. Biochem Anal Biochem 4: 234. doi:10.4172/2161-1009.1000234

phospholipids, exists as both a membrane-associated active form and a cytoplasmic latent form and plays a central role in the regulation of the synthesis of zwitterionic (PE) and acidic phospholipids [34-38]. In *B. subtilis*, the cardiolipin synthase, ClsA, which has trans-membrane α-helices at its N-terminus and amphipathic α-helices with many basic amino acid residues at its C-terminus, is located to septal and polar membranes to synthesize cardiolipin [17]. This synthase binds to membrane by its N-terminal trans-membrane helices, and then diffuses in the plane of the membrane to be captured on septal and polar membranes, which are probably rich in acidic phospholipids and their precursors, where it contributes to the formation of cardiolipin-rich domains [16,17,39]. In such domain formation, it should be noted that the transcription of *clsA* from its possible location at the edge of the nucleoid is unlikely to be important since the rate of synthesis of the protein is probably too low to generate a transertion hyperstructure unless this synthesis were to occur in a burst or to be coupled to the transertion of other proteins.

Transertion and Spirals

Transertion may help resolve the controversy over the existence of spiral hyperstructures [40-42]. Suppose a given protein tends to form a spiral due to protein–protein and protein–lipid interactions: it is then possible that, in the absence of transertion, a spiral forms whereas, in the presence of transertion, no spiral forms because this is prevented due to an unfavourable structuring of the membrane by the nascent proteins and associated lipids. Transertion might also generate a spiral if the coupled ensemble of expressed gene, nascent RNA and nascent proteins were to form a hyperstructure with a long axis parallel to the plane of the membrane.

Transertion and the Nucleoid

Transertion of a single gene not only able to pull the gene out of the nucleoid towards the membrane but also to pull out neighbouring genes that may be as far away as 90 kb [20], which is several times as long as the bacterium itself. Since such transertion may be discontinuous, it has been proposed that the result is a dynamic structuring of the nucleoid which would enable penetration of it by regulatory proteins, RNA polymerases and ribosomes [20]. Transertion of the 1000 plus *E. coli* genes predicted to encode integral inner membrane proteins [43] could therefore provide a radial force to expand the entire chromosome [7,44] and, consistent with this, many RNA polymerases and ribosomes lie between the membrane and the nucleoid [45]. Reciprocally, a relationship between transertion and peptidoglycan synthesis has recently been proposed to explain how the state of the nucleoid could help determine the width of the cell [46]. This proposal might be taken further by attributing a particular role to a transertion hyperstructure based on the *dcw* (for *d*ivision and *c*ell *w*all) cluster of genes; this conserved, 18 kb cluster, which in *E. coli* lies at the 2 min position on the chromosome, consists of 16 genes transcribed in the same direction that encode the enzymes needed for peptidoglycan metabolism and cell division [47,48]. Transertion from the *dcw* cluster could therefore create a large, transertion-based, division hyperstructure to help drive invagination at the site of division. The size and force exerted on the peptidoglycan by hyperstructures should vary with growth conditions such that, in poor media, the distribution of RNA polymerases and ribosomes would cause relatively more force to be generated both by transertion hyperstructures in general and by the division hyperstructure in particular thereby leading to narrow daughter cells. A testable prediction of this model is that the position of the *dcw* cluster on the chromosome would have an effect on peptidoglycan synthesis during cell division and affect diameter.

Transertion Problems

Because of its importance, transertion may cause problems to which cells must adapt. One problem would occur if transertion were to interfere with the structure of the chemosensing hyperstructure. The distribution of the MCPs and related proteins into many small clusters or into one giant cluster confers different sensitivities to different levels of chemo-attractants and chemo-repellents [49] and, if these distributions were overwritten by a transertion-dominated distribution, this range of sensitivities would be lost. One proposed solution is that the site of synthesis of the chemotaxis proteins is separate from the site of operation: in *E. coli*, the genes that encode these proteins are located on the chromosome so that their transertion associates them with the assembly of the flagellum; it is then conceivable that different lipid affinities of the chemotaxis and flagellar proteins lead to the chemotaxis proteins relocating from the flagellar hyperstructure(s) to the poles [50]. This proposition is supported by the diffusion of Tar-GFP from the sites where it is synthesized to the poles [51] and by the dependence of the polar location of the MCPs on neither the phospholipid composition of the cytoplasmic membrane nor the curvature of the cell poles (in fact, this location depends on the interaction of the MCPs with the trans-envelope Tol-Pal complex, which restricts the diffusion of MCP arrays [52]).

A second problem would arise if the lipid preferences of the constituents of the transertion hyperstructure led to it altering the planar, bilayer structure of the cytoplasmic membrane. The overproduction of peripheral and integral membrane proteins can indeed lead to such alterations [53-56]. A possible solution would be for an abundant structure such as the ATP synthase to have subunits with complementary lipid preferences [54] since even overproduction of all eight subunits results in morphological changes [57]. It might therefore be expected that different proteins would have complementary preferences for different lipids, which may be one reason why cells have so many different lipids.

One solution to the problems occasioned by the formation of an inappropriate transertion hyperstructure would be if cells reduced the probability of hyperstructure formation by shortening the time the nascent protein interacts with the membrane; this could be achieved by, for example, locating the membrane-interacting sequences (such as amphipathic helices) at the COOH-terminus rather than at the NH_2–terminus [19].

Transertion Perspectives

In the near future, we anticipate that transertion will be given a major role in several aspects of bacterial physiology. In the case of the structure of the nucleoid, modelling and experimental evidence increasingly favour a model in which groups of genes that are co-functional (i.e., co-expressed, or encoding products that co-assemble or that act in the same pathway) are distributed so as to organise the nucleoid into either a rosette-like or a solenoid-like structure thereby facilitating the relationship between gene expression and the rapid, efficient acquisition of coherent phenotypes [58]. It is therefore easy to imagine how the distribution of these co-functional genes may combine with transertion to structure genome, membrane and cytoplasm. Such structuring must also involve *transembly* – the coupling of transcription and/or translation to the assembly of macromolecules into complexes and hyperstructures [59]; in particular, evidence for transembly in the form of the bacterial 'nucleolus' is now compelling [60]. In the case of the cell division, a relationship with nucleoid occlusion has long been suggested [61] and attributed to transertion [4,6,7]; this is now being put in the context of a relationship between transertion and the binding of the nucleoid occlusion protein, Noc, to both DNA

and membrane [23]. In the case of the initiation of chromosome replication, a relationship with the degree of use of cellular constituents, which has been attributed to transertion [5], is being revisited as an intensity-sensing mechanism involving transertion, transembly, ion condensation/decondensation and strand separation at the origin of replication [62]. Finally, in the case of the origins of life, relatively non-specific transertion may have provided forces sufficient to resist turgor pressure and to maintain membrane integrity in the precursors of cells [19,63,64], which might help explain the importance of transertion in osmoregulation in modern cells (see above) and in determining the cell diameter [46]. This force-generating hypothesis could be tested in the former case using bacterial L-forms, which are believed to capture some of the features of early cells [65-68] and in the latter case by relocating the 2 min cluster to other positions on the chromosome and checking the cell diameter.

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