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DNA–membrane interactions can localize bacterial cell center

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Dedicated to AZ's parents, Bezal'el and Rachel Zaritsky, who passed away in their mid-90s during the summer of 2002

Abstract

In actively growing bacterial cells, the DNA exerts stress on the membrane, in addition to the turgor caused by osmotic pressure. This stress is applied through coupled transcription/translation and insertion of membrane proteins (so-called "transertion" process). In bacillary bacteria, the strength of this interaction varies along cell length with a minimum at its midpoint, and hence can locate the cell's equator for the assembly of the FtsZ-ring.

Keywords: Symmetry in cell division; Tug of war; FtsZ-ring assembly; Nucleoid occlusion

1. Introduction

In cell division of rod-shaped bacteria, the asymmetry between daughters is exceedingly low (coefficient of variation (CV) of sister cell lengths can be as small as 4%) (Errington et al., 1965; Burdett and Higgins, 1978; Koppes et al., 1978; Trueba, 1981; Grover and Woldringh, 2001). This precision is surprising since CV values of the other major cell-cycle events (cell size/age at birth, initiation and termination of chromosome replication and rate of elongation) leading to division are larger (Schaechter et al., 1962; Boye et al., 1996). The mechanism that determines the exact cell length midpoint has been debated during the last four decades (Koch and Schaechter, 1962; Koch, 1977) but is still unresolved. Several models have been proposed to explain this enigma (see e.g. Cooper, 1991; Koch, 2000), none of which seems to us satisfactory. The model currently in vogue relies on the oscillations of the MinCDE system (Raskin and de Boer, 1996; Meinhardt and de Boer, 2001; Kruse, 2002). According to this model, the oscillations of these proteins identify the cell midpoint by the difference in time spent there rather than elsewhere.

A tempting mechanism for center location is based on the so-called "Tug Of War" (TOW) concept (Koch and Holtje, 1995). This process is analogous to two teams pulling at two sides of a rope leading to a stress maximum at the midpoint. The bacterium would find its midpoint if it could discern stress levels. During growth, the peptidoglycan pulls the cytoplasmic membrane (CM) causing an uneven stress level in the CM. The mechanism proposed by Koch and Holtje (1995) relies on a delicate imbalance between the growth rates of the peptidoglycan and the CM. Since information about these rates is incomplete and other unverified assumptions are necessary for their model, it is yet to be tested. Nevertheless, we suggest that the TOW concept could be validated by the use of the interactions between the membrane and the bacterial chromosome.

2. DNA–membrane interactions

The nucleoid shapes adopted by the chromosome differ in different physiological conditions (Woldringh, 1976; Zaritsky et al., 1999, 2000). In actively growing and dividing cells, it is diffuse in form, occupying a large portion of the cytoplasm and connected to the CM, while in resting cells it is compacted in the center (Morgan et al., 1967; Van Helvoort et al., 1998). This transformation is explained by a change in the balance

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between two opposing forces that determine the dynamic nucleoid structure, i.e. compaction and expansion (Woldringh, 2002). Possible compaction forces are DNA super-coiling and phase separation between DNA and cytoplasm aided by DNA-binding proteins (Woldringh et al., 1995; Odijk, 1998), while coupled transcription, translation and insertion (“transertion”) of nascent membrane and exported proteins are considered to expand the nucleoid (Norris, 1995). Disrupting transertion annuls nucleoid expansion forces thus leading to a reduced number of DNA–membrane attachment sites and nucleoid compaction (Binenbaum et al., 1999).

3. Cell membrane as the wall of a pressure vessel

The bacterium can be modeled as an open cylinder with two hemispherical caps at its ends and constant internal pressure P_I that is generated by osmosis. Such a structure is well known in engineering as a “pressure vessel”. In the thin wall of such a structure of radius R and thickness t, two stress forces operate (e.g. Faupel and Fisher, 1981), namely, hoop, $\sigma_h = PR/t$, and longitudinal, $\sigma_l = PR/2t$. These stresses are the same throughout the whole cylindrical part of cell wall, and identical in the two half spheres ($\sigma_1 = \sigma_2 = PR/2t$). Therefore, the internal pressure alone cannot be used to locate the center of the bacterial cell. In the following, we present a model based on the additional stress due to DNA–membrane interactions.

4. The model

In our model, the nucleoid of a bacillary bacterium is considered to be a cylindrical body within the cell (Fig. 1). During the life cycle, between two successive divisions, the nucleoid is tethered to the CM with strings of DNA/mRNA/m-proteins. These strings result from the transcription/translation of genes coding for membrane (or excreted) proteins (m-proteins) and their coupled insertion to the CM by the so-called “transertion” process (Norris, 1995; Woldringh et al., 1995; Woldringh, 2002). The strings are assumed to be distributed evenly only along the cylindrical parts of the membrane and the nucleoid (see Fig. 1), in line with pole inertness (Koch and Woldringh, 1994; De Pedro et al., 2003). The DNA exerts on the CM a constant force f per string. The longitudinal stress (in addition to σ_l) due to this interaction can easily be obtained as follows.

If we denote the areas of the cylindrical envelopes of the membrane and the nucleoid as S and s, respectively, and the corresponding string densities

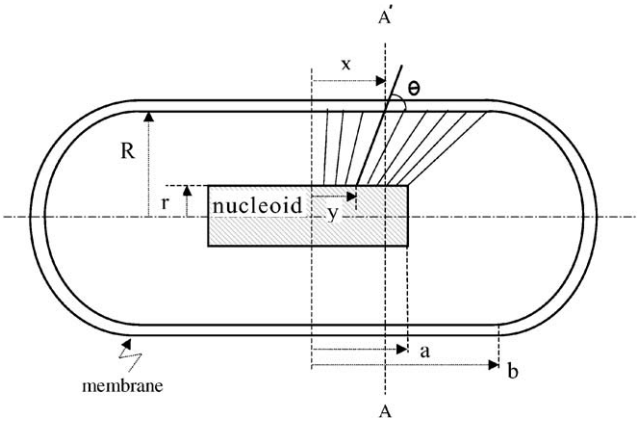

Fig. 1. The modified TOW model of a bacillary bacterium. The membrane is composed of two layers, cytoplasmic (inner) and peptidoglycan (outer). The straight lines connecting the nucleoid to the CM represent the DNA/mRNA/m-proteins strings. Strings in the first quadrant only are displayed; those not shown, present in the other quadrants, preserve the symmetry of the cell.

as N and n, then

$$n/N = S/s = Rb/ra, \tag{1}$$

where $(2b + 2R), 2R$ and $2a, 2r$ are the dimensions of the cell and the nucleoid, respectively (Fig. 1). The angle $\theta(x)$ between the string and the membrane is

$$\theta(x) = \text{arctg}[(R - r)/(x - y)], \tag{2}$$

where x varies between 0 and b, and y between 0 and a, such that the largest value of θ is $\text{arctg}[(R - r)/(b - a)]$. In the plane AA' (Fig. 1), the deviation $\Delta\sigma_l$ from the constant longitudinal stress ($\sigma_l = PR/2t$) is obtained from the balance between the forces of the strings (right-hand side of Eq. (3) below) and the internal reaction forces of the membrane ($\Delta\sigma_l 2\pi Rt$). Assuming that the strings are homogeneously and continuously distributed along the cylindrical part of the CM,

$$\Delta\sigma_l 2\pi Rt = \int_x^b N 2\pi R \, \mathrm{d}x f \cos\theta, \tag{3}$$

where t is the thickness of the CM. In Eq. (3),

$$\cos\theta = \alpha x/(p^2 + \alpha^2 x^2)^{1/2}. \tag{4}$$

Here $\alpha = 1 - a/b$, $p = R - r$ and use is made of the relation $y = (a/b)x$. The integral in Eq. (3) is straightforward, leading to

$$\Delta\sigma_1 = Nf[(p^2 + \alpha^2 b^2)^{1/2} - (p^2 + \alpha^2 x^2)^{1/2}]/(\alpha t), \quad 0 \leqslant x \leqslant b. \tag{5}$$

Fig. 2 displays the change in longitudinal stress $\Delta\sigma_l$ as a function of the distance from the center x. As can be seen, there is a minimum of stress at $x = 0$ stemming from the TOW cooperative influence of all the strings. It is thus conceivable that the hereby proposed TOW

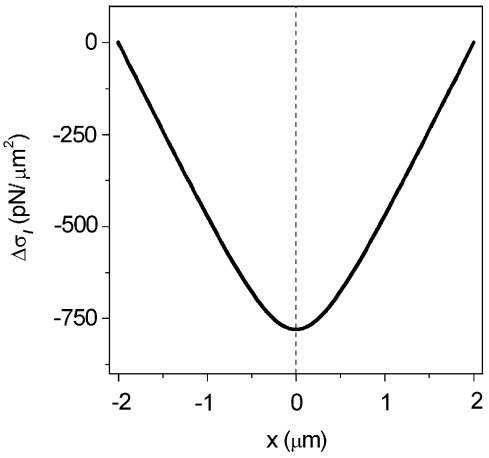

Fig. 2. The change of longitudinal stress in the CM due to DNA strings according to Eq. (5) with the following parameters: $R = 0.5\,\mu m$, $r = 0.25\,\mu m$, $a = 1\,\mu m$, $b = 2\,\mu m$, $t = 20\,nm$, $N = 10$, $f = 1\,pN$.

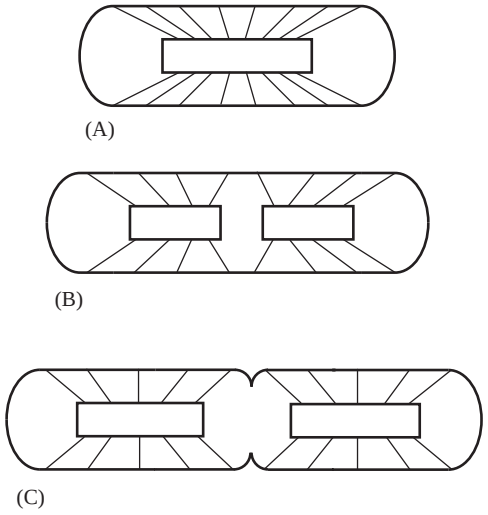

Fig. 3. A schematic transition from a minimum tensile stress to zero at the midpoint of a cell during nucleoid separation. (A) Before separation—the midpoint is under minimum stress. (B) In the initial stage of nucleoid separation—stress at midpoint is around zero. (C) After separation—midpoint stress vanishes.

model represents the mechanism for locating the midpoint of bacterial cells.

5. Implications

During nucleoid separation, the strings' directions change such that the "midpoint" passes from a minimum to a zero of tensional stress (Fig. 3). Meanwhile, two new minima are developed which serve as "midpoints" for the daughter cells. The rate of transition at midcell from a minimum to zero, being the maximal spatial rate, could actually serve as the signal to start the division process through FtsZ-ring assembly. This mechanism provides a physical foundation to the "nucleoid occlusion" model (Woldringh et al., 1990).

It was shown here that the pull, exerted by the nucleoid on the membrane through the strings, could define cell's midpoint. In a reciprocal action, the strings' pull on the nucleoid stabilizes it in the axial portion of the cell and imparts to it a cylindrical symmetry. In wider cells (dilated in their middle) however, this reciprocal pull is not sufficient to inflate the nucleoid to a volume large enough to keep this symmetry while preserving sufficiently short string lengths. What is observed in such cells is a rather different development (Fig. 2 in Woldringh, 1976; Zaritsky et al., 1999, 2000): the nucleoid is "attached" randomly to one side of the membrane by short strings and "detaches" itself from the other, thus loosing its cylindrical symmetry. Under such circumstances, the ability of an *E. coli* to place FtsZ-ring with a subsequent constriction in midcell and perpendicular to its length axis is limited, often resulting in tilted planes of division and branched cells (Zaritsky, 1977; Zaritsky et al., 1999; Zaritsky and Woldringh, 2003).

6. Concluding remarks

Our model could of course be further refined and quantified; the qualitative result should however remain valid, namely, that the TOW brought about by the DNA-to-CM transertion interaction causes unevenness in stress. The gradients thus formed, particularly the rate of transition between minimum and zero predicted at cell's midpoint during nucleoid separation, may be translated to a biochemical signal that activates FtsZ-ring formation, subsequently nucleating the division process (Nanninga, 2001). The translation may utilize proteins that appear (at least in *E. coli*) to detect decreased cell turgor or membrane tension directly by a mechanism similar to that regulating expression of porin in response to extracellular osmolarity (Blount and Moe, 1999).

The degree of precision in splitting into two daughters depends on the parameters prevailing in the cell hence it is important to measure them. The force by which each transertion string pulls the membrane and the density (and its heterogeneity degree) of these strings along cell length are examples of such parameters. It has been proposed, for example, that the density of strings decreases in partly segregated nucleoids because the frequency at which genes are expressed decreases as replication progresses towards *terC* (Woldringh, 2002). In other words, there will be regions around the nucleoid where tugging will be less intense.

Validity of our DNA-CM-TOW model does not preclude a function for the MinCDE system in discerning the cell's midpoint. Both mechanisms are likely to act synergistically.

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