INVITED PAPER

THE CELL WALL AS A MITOTIC APPARATUS IN BACTERIA

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ABSTRACT

This article reviews some of the evidence for a cell wall-DNA association in bacteria and focuses on the role of a polar cell wall in segregating replicating nucleoids in Bacillus subtilis. Evidence for a cell wall-DNA association is derived from genetic studies and from physical-chemical analyses of wall-DNA complexes. The understanding of how DNA-surface complexes function may lead to new kinds of antibiotics and to new ways to define the evolutionary relationships between procaryotic and eucaryotic cells.

INTRODUCTION

The mechanism of DNA synthesis in most well-studied bacteria has been reasonably well-defined. Furthermore, mechanisms of gene regulation are now better understood, at least in *Escherichia coli* and *Bacillus subtilis*. For some bacteria, the entire genome has been sequenced, including *E. coli* and *B. subtilis*. In spite of great advances in gene expression and molecular biology of bacteria, no one has been able to satisfactorily answer the question of how replicating chromosomes segregate into daughter cells. The now classic "replicon hypothesis" of Jacob, Brenner and Cuzin[1], first posited in 1963, attempts to answer the fundamental question of DNA partitioning in procaryotes. Jacob *et al.* assumed the replication origin to be membrane bound, and as replication of the DNA began, membrane growth occurred. The extension of membrane from a site near the middle of the cell would then provide the necessary force to segregate the replicating DNA. This was an appealing concept, and the "replicon hypothesis" of Jacob *et al.* remains important even now. Shortly after the publication of the replicon hypothesis, numerous researchers isolated and characterized DNA-membrane complexes. The attachment of DNA to the membrane was the major criterion for chromosome segregation, as envisaged by Jacob *et al.* DNA-membrane complexes were isolated from *E. coli*, *B. subtilis* and *Streptococcus pneumoniae*[2-8]. In *B. subtilis*, it has been shown that the replication origin, fork and terminus are membrane bound[5]. Lysates of *B. subtilis* subjected to various kinds of density gradient centrifugations yield DNA-membrane complexes, the DNA of which can be used to transform appropriate recipients. Funnell[8], Kolstoe[7], Sueoka[5], Kim and Firshein[6] have recently provided lucid reviews on the role of membrane in segregating chromosomal and plasmid DNAs.

One of the first concerns of the replicon hypothesis developed from results showing that membranes were fluid and unable to serve as a sufficient physical force needed to partition growing chromosomes. Another concern was that the surface expansion of both *B. subtilis* and *E. coli* were not from a central, well-defined zone, but from the addition of new wall material into pre-existing wall material over the entire cylindrical region. A third concern was the failure to isolate partitioning proteins, akin to those found in eucaryotic cells. These concerns are discussed below, accompanied by a discussion of the role of cell wall as a type of primitive mitotic apparatus.

Mohl and Gober[9], Webb *et al*[10]., and Wheeler and Shapiro[11], have recently concluded that the bacterial chromosome was attached to the tips of the cell of both *Caulobacter crescentus* and *Bacillus subtilis*. Other papers have appeared reaching the same basic conclusion that bacterial nucleoids are associated with cell poles of growing cells and cells committed to sporulation[12-16]. These papers, along with an analysis by Shapiro and Losick[17], reveal what we had previously concluded from theory and experiment. Our previous work started from the beliefs: that bacteria did not have mechanoproteins to determine their shapes; that the wall did not elongate solely between attachment points of the recently duplicated nucleoid as in the Jacob, Brenner, and Cuzin[1] replicon hypothesis; and that the pole wall was metabolically inert. This led to the surface stress theory which posits that physical forces determine the shapes of bacteria[18]. A review of evidence that mechanoproteins do not function in bacteria to cause rod-shapes has been presented[19]. Woldringh *et al*[20] and Koch & Woldringh[21] reviewed early work on autoradiography of cell wall precursors in *E. coli* and concluded that surface extension was from numerous sites. De Pedro *et al*[22] have critically confirmed that surface growth in *E. coli* was at diffuse or random sites. In *B. subtilis,* Kirchner *et al*[23] showed that the polar caps required >20 generations to turn over, whereas side walls turned over at a rate of about 50% per generation. It had been shown by others that surface enlargement in *B. subtilis* was by diffuse growth in the cell cylinder[24-25].

The reason we felt that nucleoid attachment to the poles was necessary for rod-shape growth was because this would allow the formation of a symmetrical structure leading to daughters of very nearly the same size, as is the case with *B. subtilis* and *E. coli*[26]. At first, it was suggested that the attachment would be at the junction of the pole with the side wall, but later this was modified by assuming the attachment was to the tip of the pole[19]. For *B. subtilis,* it was argued that the sites of septum formation are distinct from sites of cell elongation[24-25]. Surface elongation occurs primarily as the result of the inside-to-outside wall growth process.

Bacterial cell division is insensitive to inhibitors of mitosis of eucaryotes suggesting the absence of functional mechanoproteins, such as actins, tubulins and myosins[27]. This is in spite of the fact that several bacterial proteins possess some sequence homologies to eucaryotic cell division proteins[28]. In bacteria, there are filamentation genes, called *fts*. These genes are required for normal cell elongation, nucleoid separation and division sites (septa or constrictions). In *E. coli,* ftsZ is involved in initiation of cell constriction sites[29,30]. During cell elongation, Fts Z can be found distributed throughout the cytoplasm, but it condenses around the developing constriction site just before division[31,32]. The Fts Z contains a short sequence homology to eucaryotic γ-tubulin GTP binding sites[33]. Another gene, *fts*A, codes for a protein with an actin-like structure[34]. Another gene, *muk*B, defines a protein with a myosin-like structure[35,36]. Although some literature suggest that nucleoid segregation in eucaryotes may be driven by cytoskeletal-like proteins, there is no experimental data to suggest mechanoproteins segregate replicating DNA[19,27].

The first compelling evidence showing that there is a coupling between cell poles and nucleoid replication origins came from Sonnenfeld *et al.*[12] By use of autoradiography, following cell labeling of temperature-sensitive initiation mutants of *B. subtilis* with tritiated thymidine, it was shown that both the origin and terminus of replication were situated at or very near the cell poles. In contrast, exponentially growing *B. subtilis,* when labeled with ^{3}H-thymidine, revealed a random distribution of grains. It was also demonstrated that *B. subtilis* specifically labeled at the origin and terminus of nucleoid replication (i.e., cell poles), when treated with uncoupling agents, such as azide or carbonylcyanide-m-chlorophenylhydrazone, yielded cells with label re-distributed to the cell center[37]. The finding that loss of energized membrane causes a clustering of nucleoid toward the cell center suggests that the origin and the terminus of replication can pull away from cell poles. The "pulling" of membranes at DNA attachment sites presumably results in the formation of mesosomes[38]. Isolated cell walls of *B. subtilis* also contain DNA "contaminants" rich in markers for the origin and terminus of replication[39,40]. Finally, walls partially autolyzed to yield fragments largely of cell poles, contain most of the DNA found in original wall preparations[40]. Density gradient centrifugation patterns of lysates of cells pulse-labeled with N-[^{3}H]acetylglucosamine and then chased, revealed that a proportion of the radioactivity sedimented to a density position equivalent to that of DNA-membrane complexes[40]. The association between DNA and cell walls, including cell poles, is thus supported by genetic, ultrastructural, autoradiographic and chemical means for *B. subtilis*.
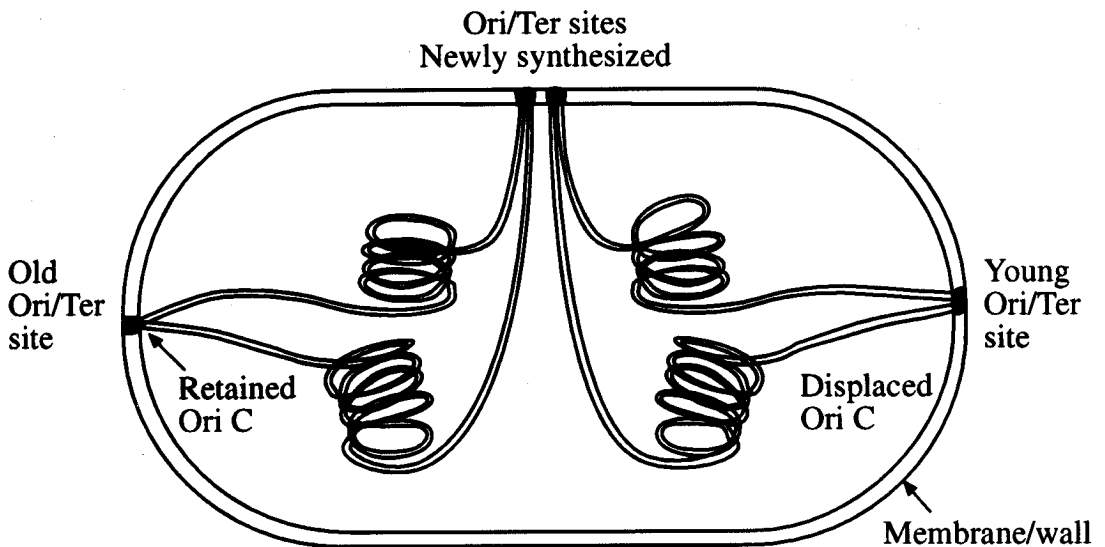
Fig.1 The renovated replicon model (RMM) for cell division of rod-shaped bacteria. The *ori* C locus (*ori*C is a gene required for initiation of DNA replication and is very near the origin of replication) binds to one cell pole, whereas the *ter* locus (*ter* is a gene found at the very end of DNA replication) binds to the other cell pole (keep in mind the binding of origin or terminus regions to pole involves membrane participation). When replication begins one sister strand remains fixed, whereas the other displaces *ter*. The DNA thus remains cell-centered in the unit (smallest or youngest) cell. This center ultimately becomes a new pole which possesses a new Ori/Ter binding site. The model accounts for the fact that daughters of *E. coli* and *B. subtilis* are virtually identical in size. Also accounted for is that *ori*C or *ter* may become displaced during a cell cycle. In addition, the RRM does not require contractile proteins for segregating the replicating chromosomes. The cell depicted above has just about completed a cycle and is near the beginning of a new cycle. Koch *et al.*[26] published the first version of the RRM. Other versions have more recently been published by Koch[19,42].

In addition to foregoing experimental reasons, the theoretical reason for assuming that during replication both origin DNA's in the replicating cell would be attached to different poles is to allow the next cell division to partition the cytoplasm evenly. Such attachment permits the replicating chromosome to serve as a symmetrical structure that would allow the terminus DNA to become centered in the cell. This would be a prelude to fixing the site of the next division. The idea was proposed that when the DNA started to replicate, one origin stayed at that pole and the other origin DNA became detached and diffused through the cytoplasm until it found the terminus DNA attached to the other end of the cell[26]. Now the terminus DNA would diffuse through the cell, perhaps sliding on the inner face of the cytoplasmic membrane. However, because the terminus DNA is part of a symmetric structure, with origin DNA attached to both of the poles, it follows that DNA replication would lead to a force that gradually centered the terminus DNA in the center of the rod-shaped cell (Fig. 1). This would fix the site of the next division at the cell's middle. These concepts could be easily added to the cell division models of Mohl and Gober[9], Webb *et al.*[10], Wheeler and Shapiro[41] and Errington[15] and goes beyond the implication of their findings. A thesis developed in this article is that the pole region, involving both the membrane and the peptidoglycan layers, are needed for regular cell division. DNA appears to bind to a membrane protein via a highly specific nucleotide sequence. How a DNA-membrane complex could in turn bind to the rigid cell pole is unknown. One possibility is that the protein which binds to DNA spans the membrane and possesses a domain capable of interaction with the wall.

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RESEARCH ARTICLES

EFFECTS OF HYDROLYSING CONDITIONS ON CHEMICAL AND SENSORY PROPERTIES OF HYDROLYSED MUNGBEAN PROTEIN

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ABSTRACT

Effects of proportion of HCl to protein, strength of acid, time and temperature in the hydrolysing reaction on chemical properties and flavour of mungbean protein hydrolysate were studied. The optimum conditions found were: ratio of protein to acid, 1:3; hydrolysing time, 6 hours; temperature, 120°C and acid strength, 6 M. At these conditions, the highest amino nitrogen, amino nitrogen to total nitrogen ratio and odor scores were accomplished.

INTRODUCTION

Mungbean protein, a by-product separated from the production of vitreous mungbean vermicelli, contains as high as 72 % (dry basis) of this essential nutrient. In addition, mungbean protein is of significant nutritional values since it is composed of all essential amino acids (Thompson, 1977). It was estimated, in Thailand, that approximately 200,000 tons per anum of mungbean seeds were used in the bean vermicelli industry. Mungbean protein which is the by-product from this industry accounted for about 27 % of the mungbean raw material. Therefore, approximately 54,000 tons per annum of this nutrient was sold as ingredient for animal feeds. This protein, if hydrolysed, may yield a flavouring product that has flavour and aroma resemble that of soybean protein hydrolysate and can be used as flavouring ingredients in food products such as soups, sauces, meat, fishery product and ready-to-eat foods.

In order that the hydrolysates would possess desirable properties, the important factors including processing conditions and selection of a raw material itself should carefully be monitored. In general, the good raw material should contain more than 30% protein (dry basis), be cheap and readily available (Grace, 1974). With significantly high content of proteins as mentioned previously, the mungbean by-product is suitable for conversion into protein hydrolysate and, thereby, efficient exploitation of such product can be achieved.

The objectives of this research were to (i) investigate the effects of mungbean protein-hydrochloric acid (HCl) ratio on chemical properties of the hydrolysed product, and (ii) study the influences of temperature, hydrolysing time and acid concentration on chemical and sensory characteristics of the product.