Ultrastructure of Deoxyribonucleic Acid-Membrane Associations in Escherichia coli

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Received for publication 1 August 1970

Areas of contact between deoxyribonucleic acid (DNA) and intracytoplasmic membrane are frequently seen in the “extra” membrane-forming strain Escherichia coli 0111a1. By examination of serial sections, it has been estimated that these DNA-membrane associations occur in at least 60% of the extra membrane-containing cells. Most of the DNA masses contained only one contact area. Several cells in which the DNA had been stretched revealed individual fibers connecting to the membrane, suggesting a firm attachment of DNA to membrane. The areas of membrane associated with DNA fibers were usually between 100 and 500 nm in diameter, although some smaller areas were seen. Electron microscopic autoradiography of cells in which the replication forks were labeled showed grains over 24% of the profiles containing a contact area, whereas there were grains over only 16% of the profiles without a contact area. Data from autoradiographs of cells in which the label was “chaired” away from the replication fork showed the reverse labeling pattern. These data indicate that the areas of contact between DNA and intracytoplasmic membranes seen in electron micrographs contain the DNA replication forks.

The occurrence of intracytoplasmic membranes has been reported in a wide variety of bacterial species. In the genus Bacillus and some other gram-positive organisms, these membranes, called mesosomes, are apparently extensions of the plasma membrane. They are regularly seen to be in contact with the deoxyribonucleic acid (DNA) mass in the cell (11). It has been assumed that the observed associations of DNA with membraneous structures correspond to the attachment of the replication fork of the DNA to a membrane component which was originally predicted by the “replicon model” of Jacob, Brenner, and Cuzin (8). This prediction has now been substantiated by several biochemical studies (3, 5, 13). In gram-negative organisms, most of which lack discrete membraneous organelles such as mesosomes, the plasma membrane is believed to provide the attachment sites necessary for DNA replication.

Recently, intracytoplasmic membranes have been demonstrated in a number of Escherichia coli strains (4, 7, 10, 12); in several of these strains, apparent associations between DNA and the intracytoplasmic membranes have been seen. Generally, the E. coli membranes are more variable in size, shape, and location within the cell than the mesosomes of Bacillus species.

E. coli 0111a1 is a strain which forms rather extensive intracytoplasmic membrane systems at temperatures of 37°C and above, but not at 30°C (12, 16). These membranes are frequently seen in contact with the DNA, and their appearance has been correlated with a slightly greater than usual amount of DNA per milligram of protein (1). The present study was done to clarify the relation of DNA and intracytoplasmic membranes in this organism. Specifically, the following questions were asked. Is the DNA actually attached to the membrane or are the two structures simply lying adjacent to one another? What is the fine structure of the attachment site? Do the visible attachment sites contain the DNA replication fork?

MATERIALS AND METHODS

Growth of cells. The organism used in these experiments has been designated E. coli 0111a1-113 (1). It was derived by nitrosoguanidine treatment of the strain 0111a1, described by Schnaitman and Greenwald (12) and requires isoleucine and valine at 37°C, but not at 25°C. In all cases, the cells were grown in Trypticase Soy Broth (TSB; BBL) at 37°C with aeration. Growth was measured by observing the optical density of the culture in a Spectronic 20 spectrophotometer (Bausch and Lomb). The cells used were either exponentially growing (3 to 5 hr of growth) or stationary phase (10 to 14 hr of growth).
Electron microscopy. The cells were fixed for electron microscopy for 2 hr at 25 C in 6% glutaraldehyde buffered in 0.1 M PO₄ (pH 7.4) and postfixed for 12 hr in 1% osmium tetroxide which was similarly buffered. They were then dehydrated in a graded ethanol series and embedded in Epon 812 by the method of Luft (9). Thin sections were cut on a Porter-Blum MT2 ultramicrotome by using a diamond knife. Serial sections were picked up on collodion-coated slotted copper grids and stained for 5 min in a saturated solution of uranyl acetate in 50% ethanol. They were then washed in boiled, distilled water and stained for 2 min in a 0.2% solution of lead citrate in 0.1 N sodium hydroxide. The sections were examined in a Hitachi HU11C electron microscope operated at 75 kv.

 Autoradiography. From a culture growing exponentially in TSB, 1.6 ml of cells was transferred to each of two prewarmed tubes containing 0.4 ml of ³H-thymidine (1 μCi/ml; 36 pg/ml). After 1 min incubation at 37 C, incorporation was stopped in one tube by the addition of sodium azide (0.001 M, final concentration), and in the other by addition of cold thymidine (200 μg/ml, final concentration). The cells in the first tube ("pulse") were fixed immediately as described above and those in the second tube ("pulse-chase") were allowed to continue growth for 20 min before fixation.

 The fixed cells were embedded and sectioned as described above. The sections were picked up on collodion-coated 200-mesh copper grids and attached to glass microscope slides by placing their edges on...
double-adhesive tape. The grids were then coated with a monolayer of Ilford L4 emulsion which was prepared by dissolving 5 g of emulsion in 10 ml of distilled water for 15 min at 45°C, placing the dissolved emulsion in an ice bath for 2 min, and then letting it sit at 25°C for 15 min more. A 1.5-inch loop of copper wire was used to pick up the emulsion and place it on the grids, by the method of Caro et al. (2). The emulsion-coated grids were then stored with a drying agent at 4°C for 5 or 8 weeks.

The autoradiographs were developed for 3 min in a 1:3 dilution of Kodak Microdol X at 22°C, fixed for 5 min in Kodak Rapid Fix, washed in two changes of distilled water, and dried. The sections were then stained for 8 min in uranyl acetate followed by 4 min in alkaline lead citrate. The autoradiographs were then examined by using a Hitachi HS 8 electron microscope operated at 50 kV.

RESULTS

Frequency of DNA-membrane contact. The technique of serial sectioning was used to estimate the frequency of occurrence of contact areas between DNA and intracytoplasmic membranes. A series of 20 or more consecutive sections was picked up on each grid. On one of the central sections from the series, profiles were selected which contained both DNA and intracytoplasmic membranes which were not in contact with each other. One such profile is shown in Fig. 1a. The selected cells were then followed in both directions from the original section, and any subsequent contact between DNA and membrane was noted. A total of 40 cells from three different cultures, two stationary and one exponential, were examined. In 60% of the cells, a clearly defined area of contact between DNA and membrane was found. Among the other 40% there were several cells in which DNA and intracytoplasmic membrane were seen very close to one another, and, in some, the DNA did come in contact with the plasma membrane. This suggests that DNA is probably associated with some form of membrane in all of the cells.

Most of the nuclear regions showed only a single area of contact with intracytoplasmic membrane. In four of the 40 cells examined, the nuclear region showed two such areas. Rarely, a cell with more than two areas of DNA-membrane contact was seen, the maximum number being seven. Figure 1 shows a series of five consecutive sections through a cell in which two...
sections reveal that the nuclear area contacts intracytoplasmic membrane in two places.

**Fine structure of the contact area.** In most cases the contact areas seen were between 100 and 500 nm in length and could be seen in several successive sections. However, a wide variation in these dimensions was noted, and, occasionally, the contact area was quite small and appeared in only one section. It is possible that this small size results from sectioning in a plane parallel to the plane of the contact area. Often, the small contact areas are found adjacent to gray areas, void of ribosomes, which are probably tangential sections of membrane. Figure 2 shows a series of sections through a contact area of the size range most frequently observed. This contact area is about 290 nm in length and can be seen in six sections. Each section is about 50 nm thick. Thus, the contact area appears to be roughly circular with a diameter of 300 nm.

<table>
<thead>
<tr>
<th>Type of profile</th>
<th>Grain</th>
<th>No grain</th>
<th>Per cent labeled</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA-membrane contact</td>
<td>23</td>
<td>72</td>
<td>24</td>
</tr>
<tr>
<td>No contact</td>
<td>44</td>
<td>236</td>
<td>16</td>
</tr>
</tbody>
</table>

**Table 1. Distribution of silver grains over intracytoplasmic membrane containing profiles: \"pulse\" experiment**

<table>
<thead>
<tr>
<th>Type of profile</th>
<th>Grain</th>
<th>No grain</th>
<th>Per cent labeled</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA-membrane contact</td>
<td>7</td>
<td>91</td>
<td>7</td>
</tr>
<tr>
<td>No contact</td>
<td>68</td>
<td>200</td>
<td>25</td>
</tr>
</tbody>
</table>

**Table 2. Distribution of silver grains over intracytoplasmic membrane containing profiles: \"pulse-chase\" experiment**
A number of cells have been observed which strongly suggest that the DNA is indeed attached to the membrane. In these cells, part of the DNA has been stretched, revealing individual fibers or groups of fibers going directly to the membrane. One of these cells is shown in Fig. 3. Most of the nuclear area in this cell is quite normal in appearance, but a small area appears to have been pulled away from the main DNA mass. The cause of the stretching is not known, but it may be brought about by rapid growth of intracytoplasmic membranes. It is not due to the fixation procedures since the rest of the cells appear normal. Figure 4 shows a section through a similar area in another cell. A large number of fibers, some of them small enough to be single DNA
helices (2 to 3 nm), apparently end at the membrane. Another cell of this type has been described in a previous publication (1). No areas of DNA stretched in this manner have been seen in which the fibers did not contact an area of membrane. This observation suggests that the DNA must be firmly attached to membrane before it can be pulled out from the nuclear mass.

**Autoradiography.** An attempt to determine whether the DNA replication fork is included in the observed contact area was made by combining the techniques of autoradiography and electron microscopy. Exponentially growing cells (54-min generation time) were placed in \(^3\)H-thymidine for 1 min to label the replication fork. Half of the culture was fixed immediately and half of it was allowed to continue growing for 20 min in media containing a high level of cold thymidine. This length of time is approximately half of the time required to replicate the entire *E. coli* genome (6), and thus the labeled stretch of DNA should have moved the maximum distance from the replication fork.

The 1-min pulse was necessary to incorporate a sufficient amount of label to do autoradiography at the electron microscopic level. Thus, the length of DNA labeled was probably several times the length of the nuclear areas. In spite of this limitation, if a part of the labeled section of DNA is actually attached to membrane, the probability of finding silver grains over those profiles in which the area of DNA-membrane association is located should be higher than the probability of finding grains over profiles from other areas of the cell. In the control experiment, where the label was chased away from the replication fork, the situation should be reversed. The results shown in Tables 1 and 2 confirm this prediction. The differences in labeling between those profiles containing contact areas and those not containing them are significant at the 90% confidence level. Figure 5 illustrates the appearance of the autoradiographs. Silver grains are localized over the DNA, and the number of background grains is negligible.

**DISCUSSION**

The intracytoplasmic membranes or “extra” membranes of *E. coli* 0111A1 are present only at temperatures above 30°C and are most extensive when the cells are approaching stationary phase (16). They are never present in all the cells of a culture. Obviously then, they are not necessary to provide attachment sites for DNA replication. However, the frequency with which these membranes are seen to be in contact with DNA and the micrographs indicating that these contact areas in fact represent firm attachments of DNA to membrane suggest that intracytoplasmic membranes, when they are present, may provide sites of DNA replication.

The resolution obtained in the autoradiographs is not sufficient to localize the DNA replication fork within the bacterial cell. However, the data showing the relatively greater labeling of those profiles containing DNA membrane contact areas compared to those in which the DNA and membrane are not in contact strongly suggest that the replication fork is included in the contact areas seen in electron micrographs. The data from the “pulse-chase” experiment show that the labeled DNA can be moved away from the contact area by further replication of the chromosome in unlabeled media.

The areas of DNA membrane contact seen in the electron micrographs are certainly larger and more complex than the single point of contact originally predicted in the replicon model. This is in agreement with data from studies with *B. subtilis* (Ramareddy and Reiter, Bacteriol. Proc., p. 52, 1970) in which as much as 20% of the genome has been found in a membrane fraction and from studies with *B. megaterium* (15) in which a complex containing 10% of the total membrane and 90% of the total DNA has been isolated. In addition, Sueoka and Quinn (14) have shown that the origin, and possibly the terminus, as well as the replication fork of the *B. subtilis* genome may be membrane-bound. It is possible that the areas of DNA-membrane contact seen in *E. coli* 0111A1 represent a complex consisting of the chromosome origin and terminus, the replication fork, and perhaps some additional points of contact to strengthen the complex during segregation of the daughter chromosomes.

**ACKNOWLEDGMENT**

This work was supported by Public Health Service research grants GM-12433 and GM-15887 from the National Institute of General Medical Sciences. J.C.S. is the recipient of a Career Development Award 5K3-GM-38,617 from the National Institute of General Medical Sciences.

**LITERATURE CITED**


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