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NOTES

Division Pattern of a Round Mutant of *Escherichia coli*

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A round mutant of *Escherichia coli*, when grown in Methocel medium, forms chains of cells and does not form tetrads. This implies that successive division planes of the round mutant are parallel rather than perpendicular. These results differ from a previous proposal that division planes in this round mutant are perpendicular to the prior division plane (W. D. Donachie, S. Addinall, and K. Begg, Bioessays 17:569–576, 1995).

When round mutants were originally isolated, they were conditional (temperature-sensitive) cells (10). Recently, Donachie et al. (9) have produced a nonconditional, stable, round mutant of *Escherichia coli*. They reported that divisions occur with successive planes of division positioned at right angles to each other. This conclusion is derived from the observation that cells growing on agar produced four-cornered tetrads, or square arrangements, at the four-cell stage. Tetrad formation is explained by the alternation of successive division planes, with a division plane occurring perpendicular to the previous division plane. This pattern differs from the parental, rod-shaped cell, where successive division planes are parallel.

If the tetrad-forming ability of round cells (due to alternation of planes of division) is real, then there are problems reconciling this growth pattern with the hoop-between-hoop model for normal cell growth. Rod-shaped *E. coli* cells are believed to grow in the side wall by the insertion of new hoops of peptidoglycan between old hoops (1–4). Hoops run circumferentially around the cell, perpendicular to the long axis. If this peptidoglycan insertion mechanism is retained in the round mutant, then it is difficult to imagine how planes of division can occur perpendicular to the previous division plane. The alternate division plane pattern would entail cutting through, rather than insertion between, preexisting hoops.

I have reexamined the growth pattern of this round mutant and find that (i) no tetrads form when the cells are grown in a viscous Methocel medium, and (ii) chains of cells are easily produced, suggesting that successive planes of division are not perpendicular.

The round mutant (*rodA*) and its parental rod-shaped cell were obtained from K. Begg (9). Any discrepancies between previous work with this mutant and the results reported here are therefore not due to genetic differences. The mutant and parental cells were struck out from the original stab, and independent colonies produced the same results.

The growth pattern of the round mutant (KJB24) and its parent (W3110) were determined using the Methocel method. Cells were grown with shaking at 37°C in Luria-Bertani (LB) broth. Exponentially growing cells were rapidly harvested in a microcentrifuge, quickly resuspended in prewarmed LB-Methocel medium, and spread on slides, using a single layer of aluminum foil to form the thin Methocel layer (5, 6). Cells were then grown in Methocel at 37°C for various periods of time. Growth was stopped by allowing the Methocel film to dry rapidly in air. The entire Methocel method has been described previously (5, 6). The fixation step and photographic emulsion cover were omitted. After methylene blue staining, photomicrographs were all taken at a magnification of ×1,000 at the microscope. The negatives were then printed electronically (all at the same relative magnification) using Photoshop software on a Macintosh computer.

The mutant cell produced chains in Methocel (Fig. 1a to k). Not all cells formed chains (Fig. 1l to m). Tetrads were never observed. If chains did not form, cells of amorphous grouping were observed. The simplest explanation for this result is that when a round cell divides, the daughter cells are able to rotate and revolve in place, thus allowing the next division to occur in a random direction. A succession of such divisions leads to the amorphous, undifferentiated grouping. During growth in Methocel the parental, rod-shaped cell consistently formed chains (Fig. 1n to p).

The growth pattern of the round mutant is not different from the parental cell, with successive planes of division occurring parallel, rather than perpendicular, to each other. This indicates that there is no major reorganization of the division process in the round mutant. Donachie et al. (9) had previously concluded that in the round mutant successive planes of division occur at right angles to each other.

A question may be raised regarding the origin of the discrepancies between the results reported here and the results of Donachie et al. (9) with regard to tetrad formation. The major experimental difference is that the round mutant formed chains in Methocel medium and tetrads when grown on agar. One historical precedent indicates that the agar method should be reexamined. This precedent concerns the proposal of the "unit cell" model (8). When normal *E. coli* cells were grown on agar it was observed that one pole appeared to remain in place. Cell elongation produced movement of the opposite pole away from its original position as the cells grew in length. Donachie and Begg (8) proposed that cells grow with a unit cell pattern, with side wall growth occurring in only one half of the cell. Later work by Woldringh et al. (12), Verwer and Nanninga (11), and De Pedro et al. (7) as well as long-term elution studies in this laboratory (1 and unpublished results) invalidated the unit cell model. Gram-negative, rod-shaped cells insert peptidoglycan all along the side wall. The side wall grows dispersively, not with a conservative, unit cell pattern. We can

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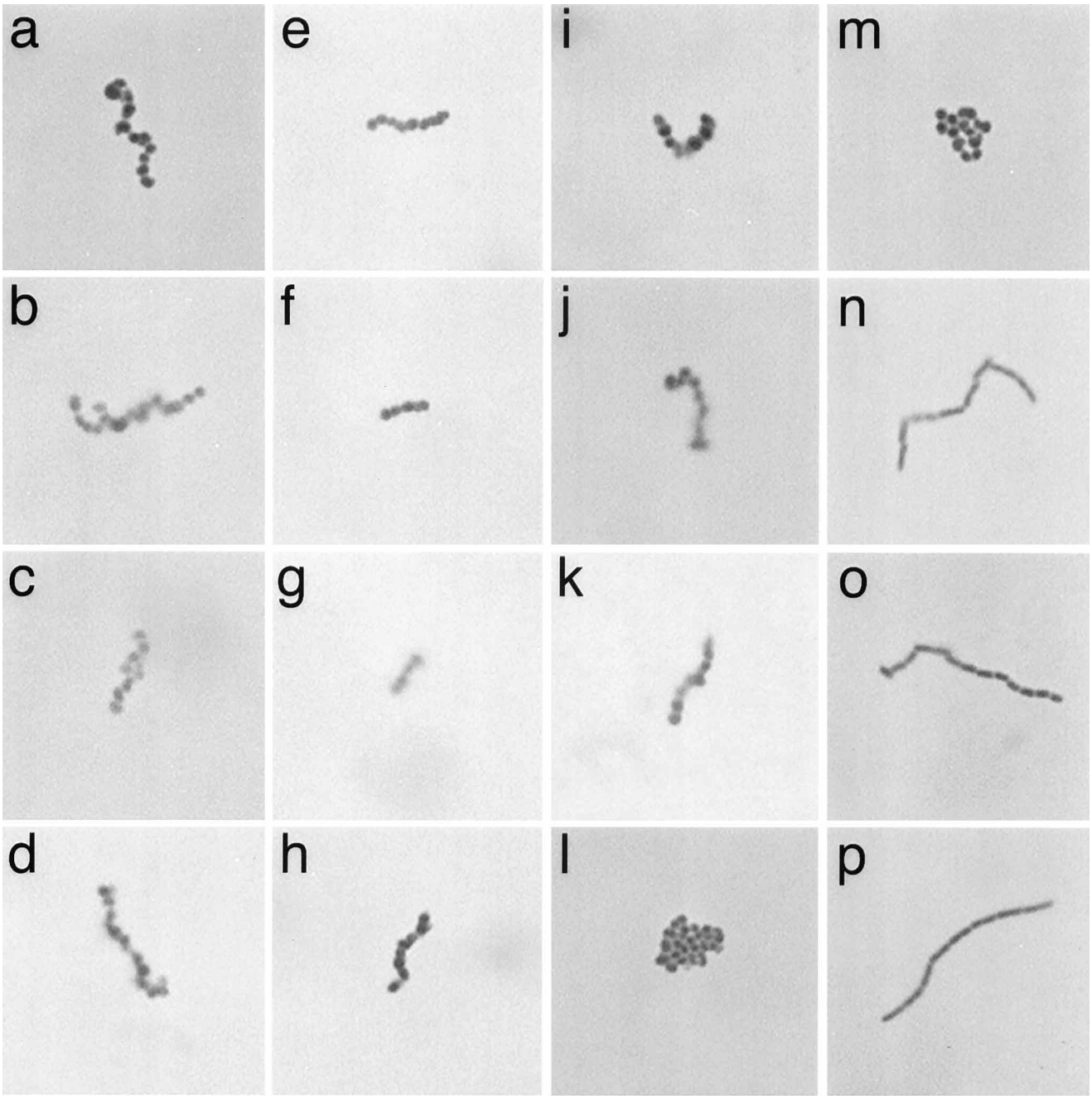

FIG. 1. Growth of the parental cell and the round mutant in Methocel medium (LB-Methocel). Representative cell groups from the negatives are shown, all at the same final magnification. (a to k) Representative chains formed by the round mutant; (l to m) representative clusters formed by the round mutant; (n to p) representative chains formed by the rod-shaped, parental cell.

now understand that if one end of a cell is preferentially stuck in agar and the other end is relatively free to move, then even cell growth through insertion of wall material over the entire length of the cell would produce movement of only one of the two poles. Thus, we should be cautious when observing growth patterns observed on agar as poles may not be free to slide on the agar surface.

If the poles of a round cell are embedded in the agar, growth between the original poles would, at the four-cell stage, produce two central or inner cells. These cells would be pushed out of line because there would be no room between the two fixed, outer cells. This movement would produce a tetrad.

One may ask why the round mutant does not form as many chains as the parental, rod-shaped cell. I offer two explanations. First, when the Methocel film is formed by pulling the Methocel over the surface of a slide with another slide, the rod-shaped cells line up in the direction of the flow of the Methocel. This alignment means that the rod-shaped cells are parallel to the surface of the slide. Growth now occurs parallel to the surface of the slide and not perpendicular to the surface

of the slide. In contrast, the round cells may not have any preferred orientation in the Methocel film. Some cells may be oriented so that the first division plane is parallel to the surface of the slide. Chains that form with growth occurring between the slide and the upper surface of the Methocel would thus be destroyed because there would be no room to extend a cell chain. A second explanation is that while the rod-shaped cells, once formed by division, may be constrained within the Methocel medium and not be able to rotate freely in all directions, a round mutant cell is not necessarily so constrained. After division, a round cell may rotate in place, leading to the formation of amorphous groupings in Methocel.

One may, if one wished, turn this argument around and say that the artifact is in the Methocel experiments. It may be argued that the Methocel, by encouraging growth along the flow of the viscous medium, encourages chain formation and prevents or eliminates the formation of tetrads. In order to accept this, one would have to propose that not only does the shape of *E. coli* change in the mutant but in addition there is a major change in the entire pattern of cell wall growth, as well as the in mode of peptidoglycan insertion. I suggest that this is an extremely complicated proposal. Using the precepts of William of Ockham ("Entia non sunt multiplicanda praeter necessitatum"), I suggest that the simplest model, with successive planes of division parallel and not perpendicular in the round mutant, is the preferred explanation. That all round cells do not form observable chains should not detract from the two main observations: (i) round cells do not form tetrads in Methocel, for none are ever seen, and (ii) round cells do form chains in Methocel.

To summarize, round mutant *E. coli* cells grow in a manner similar to the parental cell, with successive division planes parallel to each other.

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