

# Cell Surface Patterning and Morphogenesis: Biogenesis of a Periodic Surface Array During *Caulobacter* Development

JOHN SMIT and NINA AGABIAN

Department of Biochemistry, University of Washington, Seattle, Washington 98195

**ABSTRACT** Shape changes, extended processes, and other surface elaborations are associated with cellular differentiation, and the cell membranes involved with these developmental changes often are reshaped without a major alteration in biochemical composition. *Caulobacter crescentus* produces a hexagonally-packed periodic surface layer that covers the entire cell and further, mimics some of the membrane-mediated changes of higher organisms by forming a membranous stalk during its distinctive life cycle. Growth of the surface layer was examined during the cell cycle by treating synchronously growing cells with surface layer antibody, continuing growth, and then labeling for electron microscopy with a protein A-colloidal gold conjugate. Three regions of distinctive surface array biogenesis were resolved. The periodic surface layer on the main cell body was enlarged by insertion of new material at numerous uniformly distributed points. In contrast, the surface layer on the stalk appeared as entirely new synthesis. In examining growth of the stalk in subsequent generations, we noted that growth of stalk surface persisted at the stalk-cell body junction. The region of cell division also showed a pattern of entirely new surface layer production at late stages in division, similar to the stalk. The immunocytological method also facilitated a careful examination of stalk initiation and growth. Although initiation was under precise temporal and spatial regulation, the rate of stalk elongation was variable from cell to cell and apparently no longer under cell cycle control.

The similarity of surface layer biogenesis on the stalk and the site of cell division may be a significant reflection of other events occurring at the cell pole. A model suggested by this and other studies that can account for the temporal pattern of polar morphogenesis is discussed, as is the potential relationship between the geometrically ordered surface array and the formation or maintenance of the stalk.

In the differentiating bacterium, *Caulobacter crescentus*, morphogenesis is confined to the polar regions of the cell in a process of patterning events that are spatially and temporally coordinated with other developmental changes. As *Caulobacter* progresses through each cell cycle, a motile swarmer cell develops into a nonmotile stalked cell by the site-specific outgrowth of a stalk. During this transition, there is a simultaneous loss of swarmer cell surface structures, which include the polar flagellum and several pili (1, 2, Fig. 1). A new swarmer cell is produced by localized synthesis of these structures at the developing cell pole.

Superimposed on this complex differentiation pattern is the continuous production of a periodic surface array, composed of at least three proteins (3). The surface array of *Caulobacter* is structurally different from the more densely packed arrays

of other bacteria and is found over the entire *Caulobacter* cell surface, including the stalk (Fig. 2). Surface arrays appear to be ubiquitous in procaryotes, but little is known about their biosynthesis, export, and assembly, or their importance to the cell (4). Because morphogenesis in *Caulobacter* is essentially a problem of membrane differentiation and specialization, the relationship to surface array biogenesis was examined. Morphogenesis in this instance includes organelle development, the orientation of the division plane, and the production of a stalk. The stalk is composed of and continuous with the Gram-negative membrane layers that encompass the entire cell (2). Biochemical differences in the stalk relative to the remainder of the cell have not been detected (5).

In an effort to understand these related membrane processes, we examined the pattern of biogenesis of the *Caulobacter*

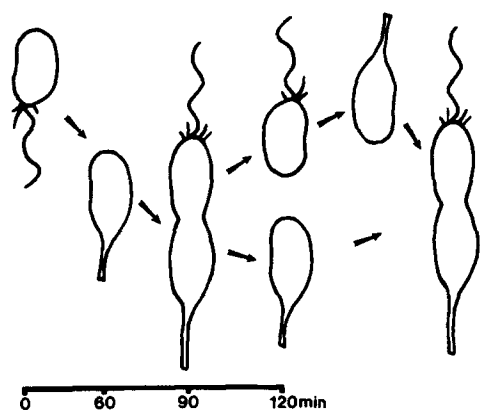


FIGURE 1 Diagrammatic life cycle of *Caulobacter crescentus*. Note that the swarmer cell at left has a flagellum and several pili that are lost during the transition to a stalked cell. Also note that after division the stalked cell immediately reinitiates the reproduction process, whereas the daughter swarmer cell must first develop to the stalked cell stage. The time line indicates the approximate length of time required for the morphological stages to appear in a synchronous population growing in HMG medium at 30°C.

surface array using immunocytological methods. These studies have elucidated unique growth zones in the polar regions of the cell surface whose appearance is correlated with the cell cycle. The pattern of labeling observed points out that, for membrane layers that are continuously produced, localized regions of differential assembly can contribute to the morphogenetic process.

## MATERIALS AND METHODS

### Bacterial Strains and Growth Conditions

For all labeling experiments described, *Caulobacter crescentus* strain CB15 (ATCC 19089) was used. Cells were grown in Hutner's minimal glucose medium (HMG) (6) at 30°C on rotary shakers. Synchronously growing populations of swarmer cells were prepared using the Ludox procedure (7).

### Preparation of Surface Array and Production of Antisurface Array Antibody

Surface array was isolated by differential centrifugation from cultures of *C. crescentus* strain 15NY106 (8) grown to high density, as described previously (3). 15NY106 sloughs off pieces of array that are large and more easily isolated; however, the composition of surface array from this strain is thus far indistinguishable from that of parent strain CB15. Total protein content of these preparations was estimated using the Lowry assay (9), with bovine serum albumin as a standard. A New Zealand White female rabbit was immunized with surface array (containing 1 mg of protein) in complete Freund's adjuvant by intramuscular and subcutaneous injections. After 21 d, a series of three subcutaneous injections containing 0.35 mg of protein in incomplete Freund's adjuvant were given at 7-d intervals. A maximal response, as judged by double diffusion analysis (10), was obtained on days 53 and 60. Partial fractionation of the immunoglobulin was achieved by precipitation from sera with 50% saturated ammonium sulfate and dialysis against phosphate-buffered saline. The concentration of immunoglobulin was standardized at approximately twice that of the original serum.

### Preparation of Protein A-Colloidal Gold Label

The protein A-colloidal gold complex (pA-CG) was prepared using a method similar to that of Roth et al. (11). In a typical preparation, 600  $\mu$ l of 1% chloroauric acid (50% Au content, Fluka A. G., Basel, Switzerland) was added to 48 ml of H<sub>2</sub>O. 0.8 ml of 0.2 M K<sub>2</sub>CO<sub>3</sub> was added, followed by 400  $\mu$ l of a solution of phosphorus in diethyl ether (4 parts diethyl ether and 1 part saturated phosphorus in ether). The mixture was shaken and allowed to stand at room temperature for 15 min. The mixture was heated to boiling for 5 min, cooled to room temperature, and lost volume was restored with H<sub>2</sub>O. Protein A (1 mg per ml in H<sub>2</sub>O) was placed in a polyethylene bottle and the freshly prepared colloidal gold was added

at a ratio of 20 ml/mg protein A. The mixture was shaken, allowed to stand for 3 min, and a 1/20th volume of 1% polyethylene glycol (average mol wt 20,000) was added. The polyethylene glycol was filtered (0.45  $\mu$ m, Millipore Corp., Bedford, MA) immediately before use. The stable pA-CG conjugate thus formed was centrifuged at 150,000 g for 90 min. The supernatant fraction was immediately removed by aspiration and the pellet was suspended in 0.05 M Tris (pH 7.0), 0.15 M NaCl, 0.05 mg/ml polyethylene glycol (average mol wt 20,000) and 0.003 M NaN<sub>3</sub> (0.75 ml/mg protein A). This final preparation was stored at 4°C.

### Protein A-Colloidal Conjugate Labeling of Antibody-treated Cells

Synchronously growing populations of swarmer cells were prepared as described above and adjusted to about  $4 \times 10^9$  cells/ml in HMG medium. Antisurface array antibody was added to the cells at a ratio of 40  $\mu$ l antibody/ml of cells, and the mixture was incubated for 10 min on ice. Unbound antibody was removed by three cycles of centrifugation and suspension in cold HMG medium. The cells thus treated were permitted to initiate synchronous growth by incubation at 30°C in HMG medium. At 0-, 30-, 60-, 90-, and 120-min intervals, 1-ml portions were removed and treated with 25  $\mu$ l of pA-CG. After 5 min of incubation at room temperature, unbound pA-CG was removed by three cycles of centrifugation in H<sub>2</sub>O. The final cell pellets were suspended in 50  $\mu$ l of H<sub>2</sub>O. The unfixed and unstained cells were immediately applied to Formvar-film copper grids that had been stabilized by vacuum-deposited carbon and rendered hydrophilic by vacuum deposition of a layer of silicon monoxide (12). Excess cells were removed by touching grids to filter paper. When negative staining was desired, 2% ammonium molybdate (pH 7.5) was mixed with equal volumes of cell suspension before preparing grids. The preparations were examined in a Philips 201 electron microscope, operated at 60 kV.

For some experiments, synchronous swarmer cells were allowed to differentiate into stalked cells by incubation at 30°C for 60 min before the antiarray antibody treatment. The antibody treatment was that described above, except that the incubation with antibody was done at room temperature. Incubation of these cells was continued and pA-CG labeling was done at 120 or 180 min. In one set of control experiments, synchronous swarmer cells were treated with antibody at 0 min, grown for 60 or 120 min and, before the pA-CG label, a second cycle of treatment with antiarray antibody and subsequent washes was done. Negative control experiments, done by treating cells with nonimmune rabbit serum or antiserum directed to the flagellum (13) or the pili of *Caulobacter* (14), demonstrated that nonspecific labeling of the cell surface was virtually nonexistent (data not shown).

A microfuge (Brinkmann 5412, Brinkmann Instruments, Inc., Westbury, NY) was used for all centrifugation steps done after a growth cycle of synchronous swarmer cells was started. In all cases of labeling and washing, the interruption in the cell cycle required to perform those steps was not considered when assigning the age of the synchronously growing cells. The amount of error introduced in this way was not significant for any of the conclusions reached.

The method described for labeling the cell surface worked well and with minimal disruption of normal events of the cell cycle. The pA-CG conjugate was a well-dispersed labeling reagent with a minimal number of clusters of gold particles. The electron-dense gold particles were very small (~50 Å diameter) and uniform. The combination of both small size and the absence of clusters permitted high-resolution analysis and produced a spatial clarity not possible with other immunological techniques. Labeling cells with antibody and later visualizing antibody with pA-CG permitted the cells to be grown without being encumbered by a considerable amount of particulate gold. Under these conditions, the cells appeared essentially unaffected, as demonstrated by normal patterns of development and morphogenesis, as seen by light microscopy (data not shown). The only effect noted was a loss of motility of the synchronous swarmer cells, almost certainly due to a significant amount of antibody activity directed against the flagellum in the antiarray antibody.

### Gel Electrophoresis and Western Blotting

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) was performed as previously described (15). For most experiments, a gradient gel of 10-17% acrylamide was used. Antibody binding activities of the proteins separated in this manner were detected by using the "Western blot" procedure, where proteins are electrophoretically transferred to a sheet of nitrocellulose after SDS PAGE. The immobilized protein bands are then treated with antibody. The details of the transfer process, antibody treatment, and subsequent washes are similar to those described by Burnette (16). In our hands, enough protein remained in the polyacrylamide gel to be stained by Coomassie Brilliant Blue. This facilitated distinguishing between protein bands that did or did not bind antibody.

Detection of those protein bands that bind the rabbit antibodies was accom-

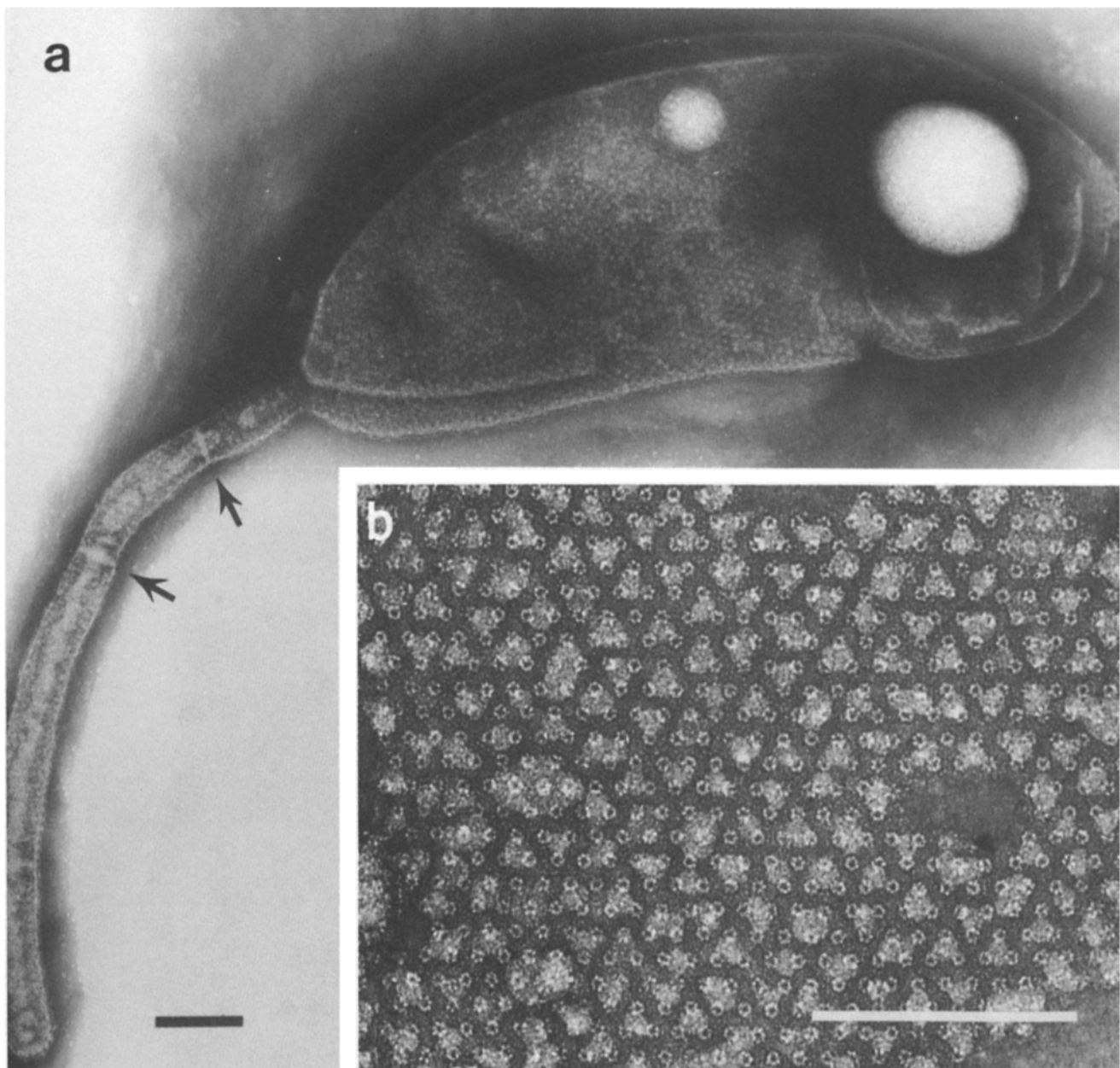


FIGURE 2 (a) Negative staining of *Caulobacter crescentus* CB15 and the periodic surface array. The surface array appears as a repeated structure covering the entire surface of a lysed stalked cell, including the stalk; inner and outer membranes can be discerned as well as several crosswalls in the stalk (arrows). The electron-transparent spheres are granules of poly- $\beta$ -hydroxybutyrate, a carbon storage product for this bacterium (28)  $\times 67,000$ . (b) Isolated surface array. At higher magnification the precisely ordered, six-member rings are visible. The amorphous shapes irregularly interspersed in the lattice are vesicles of the outer membrane that remain attached to the isolated structure (3). In both micrographs the negative stain is ammonium molybdate. Bars,  $0.2 \mu\text{m}$ .  $\times 200,000$ .

plished by treatment of the blots with peroxidase-conjugated goat anti-rabbit immunoglobulin (Antibodies Inc., Davis, CA) at a 1:1,000 dilution. Western blots were incubated for 2 h and washed several times as described (16). For the peroxidase-catalyzed color reaction, the blots were suspended in 40 ml of 0.05 M Tris (pH 7.5) and 0.15 M NaCl (TBS) to which 10 ml of 4-chloro-1-naphthol solution (3 mg/ml in methanol) and 50  $\mu\text{l}$  of 30%  $\text{H}_2\text{O}_2$  were added. The reaction was terminated after  $\sim 10$  min by washing with TBS. The stained blots were immediately photographed, as the color fades with time.

## RESULTS

Three distinct areas of surface array biogenesis were detected when antiarray-treated synchronous swarmer populations were grown and then labeled with pA-CG at selected times in the cell cycle. New array material was inserted at numerous sites

over the cell body, as shown by a progressive drop in the density of colloidal gold particles on the cell surface during the cell cycle and the absence of areas of significant size that were devoid of particles (Fig. 3 and 4).

The pattern of deposition of surface array on the new stalk, which develops during the swarmer to stalked-cell transition, contrasted strikingly to that seen over the cell body. When swarmer cells were treated with antibody, grown to the stalked-cell stage, and then labeled with pA-CG, the stalk was virtually unlabeled (Fig. 3 c), indicating that no preexisting array materials were used in the production of the stalk structure. Even very early stages of stalk growth showed that absence of label. For some cells examined after 30-min growth time, the labeling

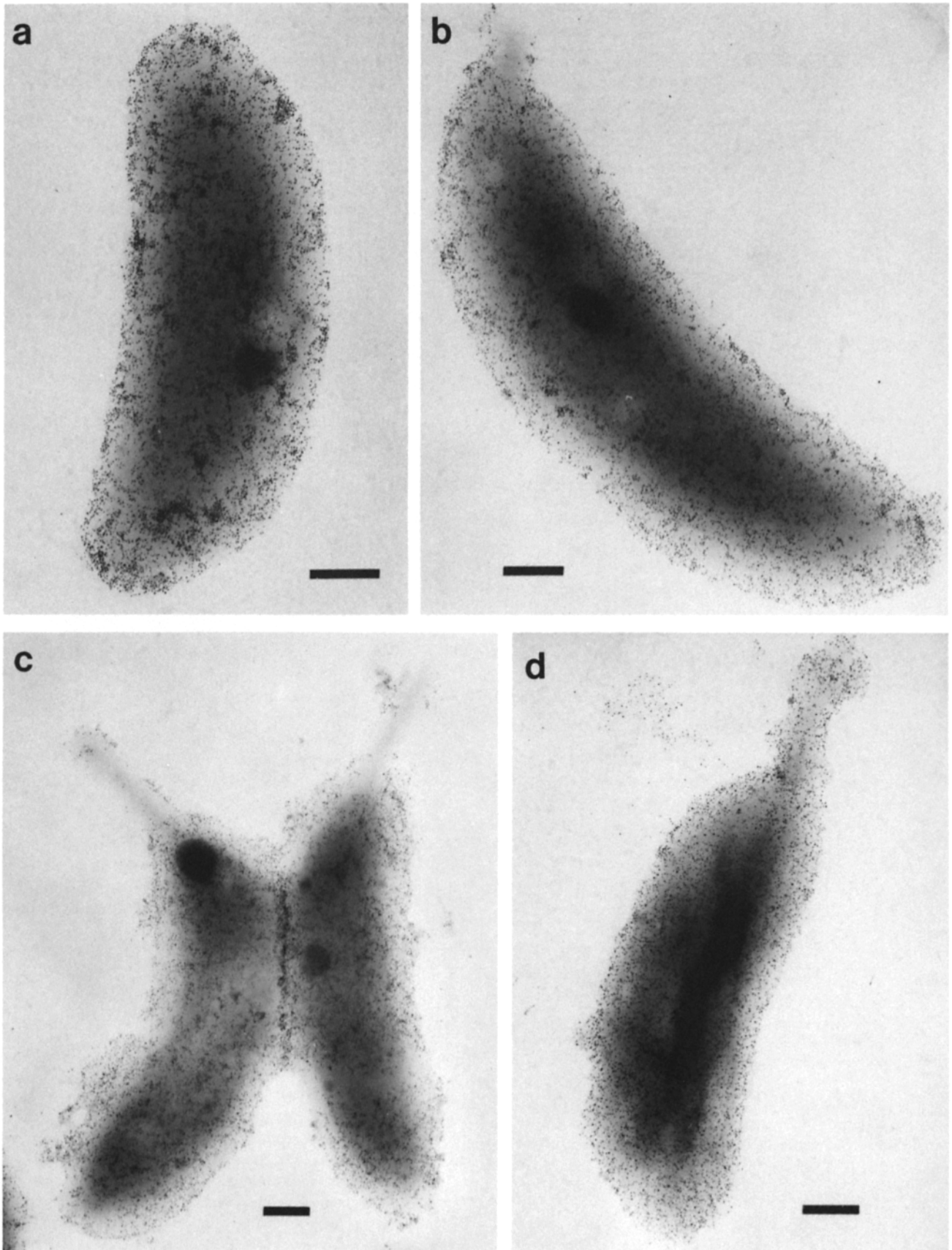


FIGURE 3 Protein A-colloidal gold labeling during growth of a synchronous cell culture. A synchronous population of swarmer cells was labeled with antisurface array antibody as described in Materials and Methods. At selected times during the cell cycle, a portion of the culture was treated with pA-CG to determine the location of bound antibody. (a) Swarmer cells (0 min of growth), (b) 30 min of synchronous growth, (c) 60 min of synchronous growth (stalked cells). (d) This is also a 60-min sample; however, prior to pA-CG treatment the cells were again treated with the antibody. Bars, 0.2  $\mu\text{m}$ . a,  $\times 62,000$ ; b,  $\times 53,000$ ; c,  $\times 41,000$ ; d,  $\times 49,000$ .

pattern is essentially diagnostic in determining where the stalk will appear (Fig. 3*b*).

The surface array of *Caulobacter* extended over the entire surface of the cell, including the stalk, but the possibility remained that array was deposited on the stalk after the underlying structure was formed. The presence of array on newly formed stalks was confirmed by re-treating synchronous stalked cells with antiarray antibody before the pA-CG steps. This control experiment also demonstrated that the stalk has surface layer components present that labeled at a density comparable to that of the main cell body (Fig. 3*b*).

The third area of growth distinguished by this technique was the region where cell division occurs. When antibody-treated cells were labeled with pA-CG after 120 min of synchronous growth, many cells had already divided, but of those predivisional intermediates that remained, all showed a region devoid of label at the site of new pole formation (Fig. 4*b*). Apparently here, too, an area of entirely new surface material was formed. An antibody re-treatment experiment, similar to that used with stalked cells, confirmed that surface array components were present at the site of division (data not shown). The region devoid of colloidal gold label persisted after division as a bare spot on the new poles of the stalked and swarmer cell progeny. The persistent integrity of the unlabeled region (Fig. 4*c-e*) indicated that the labeling pattern obtained was probably not caused by "stretching" the existing surface layer of predivisional cells, thereby reducing the density of antibody molecules, but rather was a region where differential biogenesis of surface array had occurred.

The deposition of entirely new surface material at the division site was restricted to the last stages of the division process. Cell populations examined after 90 min of growth have already determined the site of the division plane as evidenced by the first stages of constriction in that area. But, at 90 min these regions of constriction are uniformly labeled (Fig. 4*a*), in contrast to those labeled at 120 min (Fig. 4*b*). This suggests that the surface components do not have a role in determining where the division plane is placed.

The labeling procedures permitted us to distinguish between the mechanisms of stalk elongation. Elongation could occur by growth from the distal tip, or from a region at the base of the stalk, by diffuse growth along the entire length of the stalk, or even as a result of collapse of the end of the bacterium, thus largely drawing upon preexisting membrane material. Determining the actual mechanisms is relevant not only to the initial production of the stalk but also for future generations, in which the stalk continues to elongate. A synchronous cell population was grown for 60 min before antibody treatment, resulting in antibody bound to the first generation stalk. After 120 or 180 min of growth, pA-CG labeling clearly showed an area devoid of label at the base of the stalk, implicating this region as the stalk growth zone (Fig. 4*c*). This result confirmed the conclusion of Schmidt and Stanier (17), who studied the stalk outgrowth by <sup>3</sup>H-glucose labeling and electron microscope autoradiography. <sup>3</sup>H-glucose labels other membrane components in addition to the surface array, and therefore comparison with these earlier findings provides additional evidence that the biosynthetic events occurring on the cell surface reflect biosynthetic events in the underlying membranes of the stalk.

It was not possible to achieve reciprocal labeling patterns by reversing the labeling procedure. That is, we attempted to block preexisting surface layer by treatment with unlabeled antibody, continue growth, and label with colloidal gold conjugated directly to antiarray antibody, but it was not possible to com-

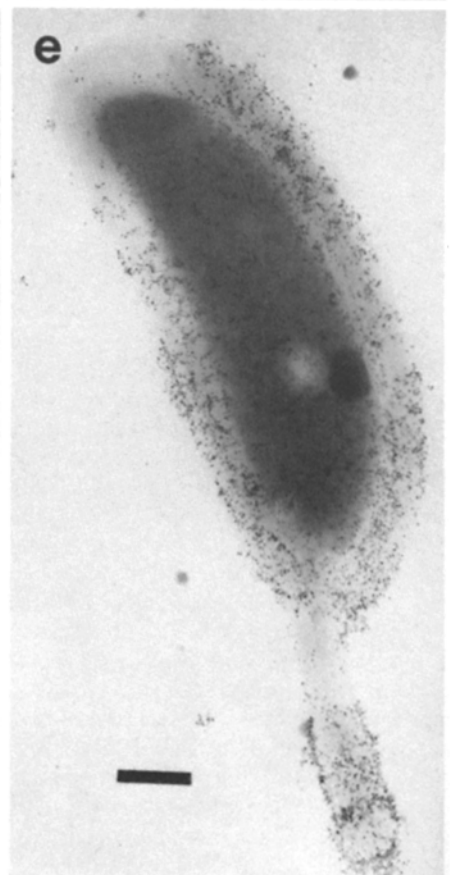
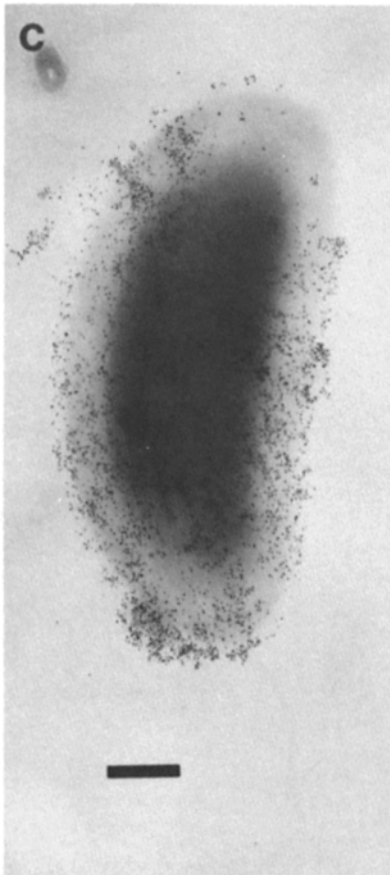
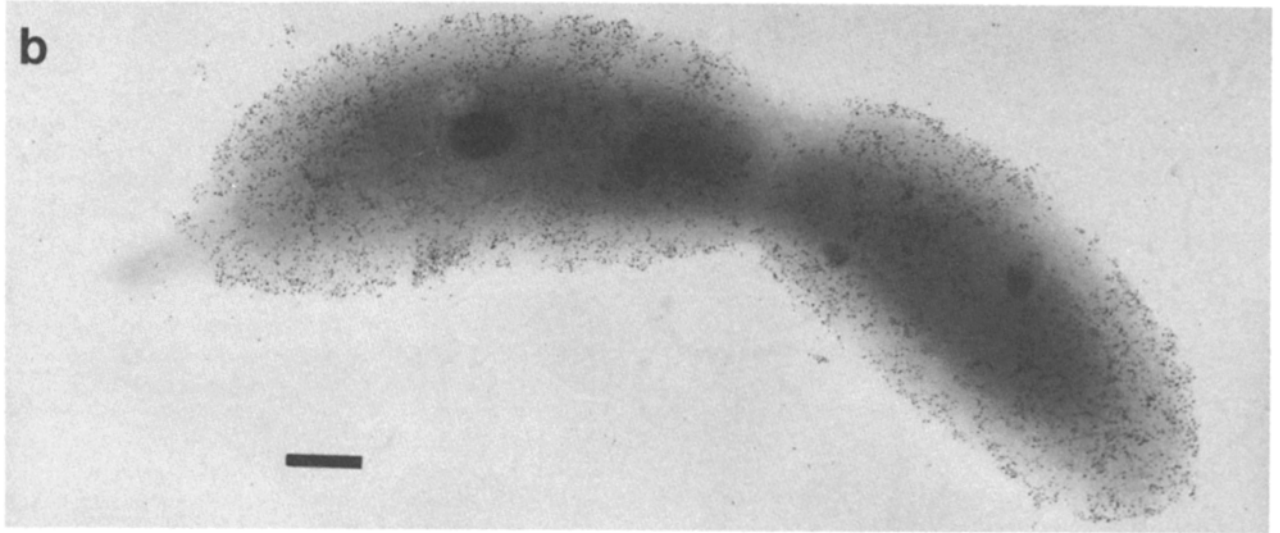
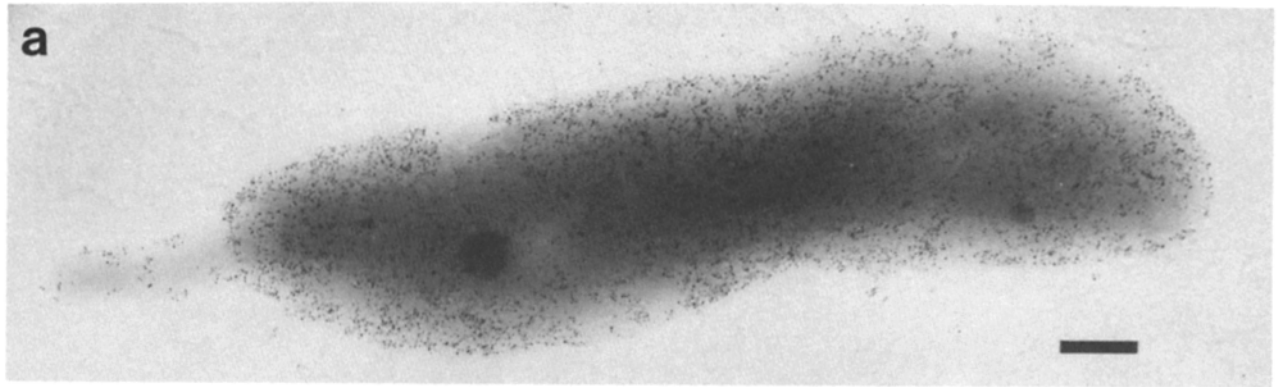
pletely prevent binding of the labeled antibody. Thus, areas of new growth could not be readily distinguished (data not shown). These results were comparable to those of a similar experiment in the analysis of proteins exposed on the surface of *Salmonella typhimurium* (18) and may indicate the general impracticability of completely masking antigens present on surfaces at high density with homologous antibody.

It has also not been possible to label the cell surface with colloidal gold and simultaneously reveal the surface array by negative staining. In general, it is very difficult to routinely visualize the surface array on intact cells by negative staining. This limitation seems even more pronounced when immunoglobulin molecules are attached to the surface. Thus, precise details of the manner in which new components are incorporated into the surface layer could not be studied. It was possible, however, to record images that indicated that the colloidal gold label was bound very close to the cell body (Fig. 5), indicating that the data recorded from unstained cells were not the consequence of a substantial, unaccounted-for capsular layer.

It was possible to precisely compare the temporal relationship between stalk growth, cell growth, and division by using the increased resolution provided in these labeling experiments. All cells in a synchronous population had formed stalks of some size by 60 min in the cell cycle. Thus, the appearance of a stalk does seem to occur in a synchronous manner. The length, however, of this first generation stalk was variable. Some cells continued to elongate their stalks during the predivisional intermediate stage, whereas others did not. When examined after cell division, most of the stalked cell progeny had elongated their stalks, but again the amount of growth was variable. Moreover, there was a distinct proportion of stalked cells whose stalks had not increased in length by the time they had become "second generation" predivisional intermediates. Thus, it may be that the process of initiating stalk synthesis is tightly coupled to other cell cycle processes, such as DNA replication (19), but, once the stalk is formed, further elongation of the stalk is under an independent, more loosely regulated pattern of control.

The antiarray antibody used for these experiments was characterized by the western blotting procedure. The surface array preparation used as immunogen was composed of three predominant proteins that are thought to be responsible for the repeated array appearance (3). In addition, some free flagella, composed of a hook protein and two flagellin polypeptides (20), were usually present. Enough flagella were in the immunogen to elicit an antibody titer adequate to immobilize the flagella of swarmer cells in these experiments. As expected (Fig. 6), the western technique showed significant activity to the 130K and 20K polypeptides. The western technique is extremely sensitive to minor antibody activities; thus numerous other peptides, presumed to be membrane proteins, were also labeled, many of which were too minor to be seen by Coomassie Brilliant Blue staining (Fig. 6). The unexpected result was an absence of activity in response to the 74K protein. Subsequently, it was learned that the conspicuous lack of labeling was also true for western blots using antibodies raised against other antigen mixtures containing 74K, such as whole cells (data not shown). Apparently, the 74K is a very poor immunogen in rabbits. Thus, the pattern seen does not reflect deposition of this protein during cell growth.

The consensus of the data therefore indicates that the labeling pattern observed represents antibody binding to the 130K and 20K proteins. We cannot entirely rule out the possibility that there is a thin layer of a nonprotein component covering



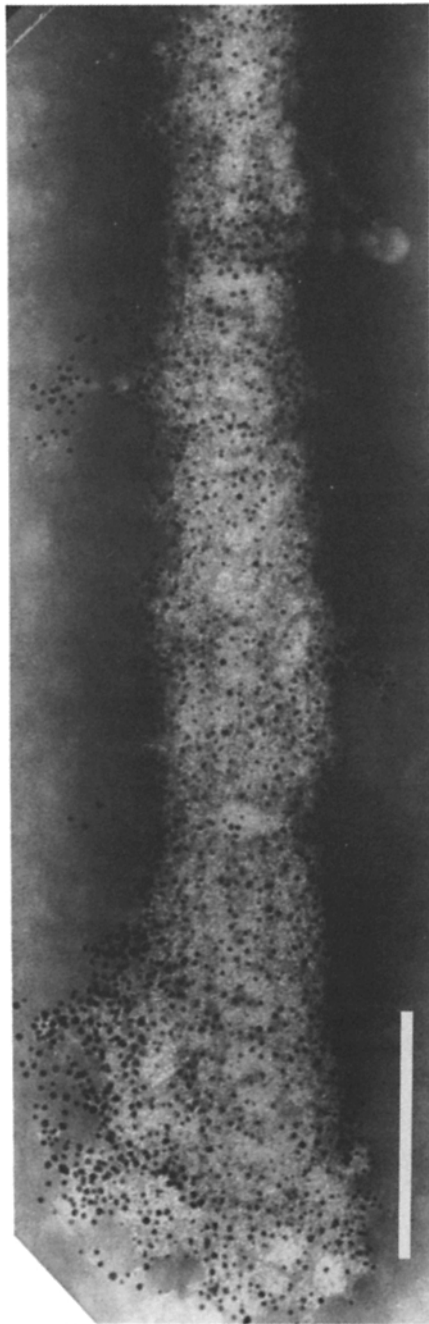


FIGURE 5 Negative-stain microscopy of a stalk that has been labeled with the antiarray antibody/pA-CG procedures. Elements of the periodic surface array cannot be easily discerned, but note that the colloidal gold label is in close contact with the stalk, discounting the possibility that a thick capsular layer accounts for the labeling pattern observed. Bar, 0.2  $\mu\text{m}$ .  $\times 161,000$ .

the visible array elements whose deposition accounts for the labeling pattern observed. Such a component might escape detection in the western assay. However, two-dimensional crossed immunoelectrophoretic analysis also revealed no additional antigens in an amount comparable to that produced by 130K (data not shown). Such a relative quantity would seem necessary for a component that covers the entire surface.

## DISCUSSION

The pattern of surface array biosynthesis functionally delineates three regions of the *Caulobacter* cell surface, apparently the result of differential assembly of uniform components at different periods in the cell cycle. The surface layer on the cell body grew by diffuse intercalation, resulting in a mixing of new and old material over the entire surface. An exception to this labeling pattern was found in the surface layer of the stalk and new cell poles formed by division. Surface array was deposited on the stalk as entirely new material, probably emanating from a distinct region on the cell surface (the future stalk-cell junction). A similar localized region of array export and assembly occurred at the site of cell division during the last stages of cell separation.

Membrane growth by a diffuse intercalation process was not an unexpected finding for a Gram-negative bacterium. In *Escherichia coli* and *Salmonella typhimurium* it has been shown that lipopolysaccharide, phospholipids, and membrane proteins are inserted at numerous sites that are distributed uniformly over the cell surface (18, 21, 22). Export of these components occurs through membrane adhesions (18, 21), transiently stable connections between the inner and outer membrane (23). It is tempting to speculate that the *Caulobacter* surface layer components are exported through similar adhesion sites. Although this may explain how components reach the cell exterior, it does not explain how the new material is incorporated into the existing, rigidly organized network of the surface array without distorting the structure. It is possible that new components are added as "rings" of array (that is, chains formed by connecting the six-member structures [Fig. 2] around the circumference of the cell) at random locations along the length of the growing cell. The spatial resolution of the labeling technique may not permit visualization of the fine points of such a process.

The concept of enlarging the surface layer by inserting loops or rings of the periodic structures, with the subunits originating from sites of adhesion between the inner and outer membrane, could also be extended to explain surface layer deposition on the growing stalk. One might expect this region of membrane adhesion to be a more stable or permanent structure, perhaps even extending through numerous generations as the stalk continues to grow. On the basis of evidence of proximal stalk growth, whatever the nature of the "growth zone," it is presum-

FIGURE 4 Protein A-colloidal gold labeling during growth of a synchronous cell culture. For a and b, cells were treated with antibody as swarmer cells (0 min) and at the indicated times the pA-CG labeling was performed: (a) 90 min of growth. Note that the region of cell constriction, which indicates a future division plane, has a gold particle density comparable to that of the rest of the cell body. (b) After 120 min of growth. Note that, in addition to the unlabeled stalk, a region devoid of gold particles forms at the constriction site during the last stages of division. For c-e, synchronous swarmer cells were grown for 60 min, producing synchronous stalked cells, before antibody treatment. pA-CG labeling was done at the times indicated. (c) 120 min. Note the bare region, devoid of pA-CG, on this swarmer cell that persists after division. (d) 180 min. This cell is one complete generation removed from the original stalked cell population and therefore demonstrates a bare cell end, resulting from division, and a bare, newly formed stalk. (e) 180 min. This cell is also one generation removed from the time of antibody treatment, but is the stalked cell progeny of the division, demonstrating the bare division plane and second generation stalk growth. Note that growth of the stalk is occurring at the stalk-cell junction. Bar, 0.2  $\mu\text{m}$ . a and b,  $\times 49,000$ ; c-e,  $\times 48,000$ .

ably an annular region surrounding the stalk at its base. In sum, one could postulate that the formation of the stalk surface layer is a special variation of what is occurring over the majority of the cell surface.

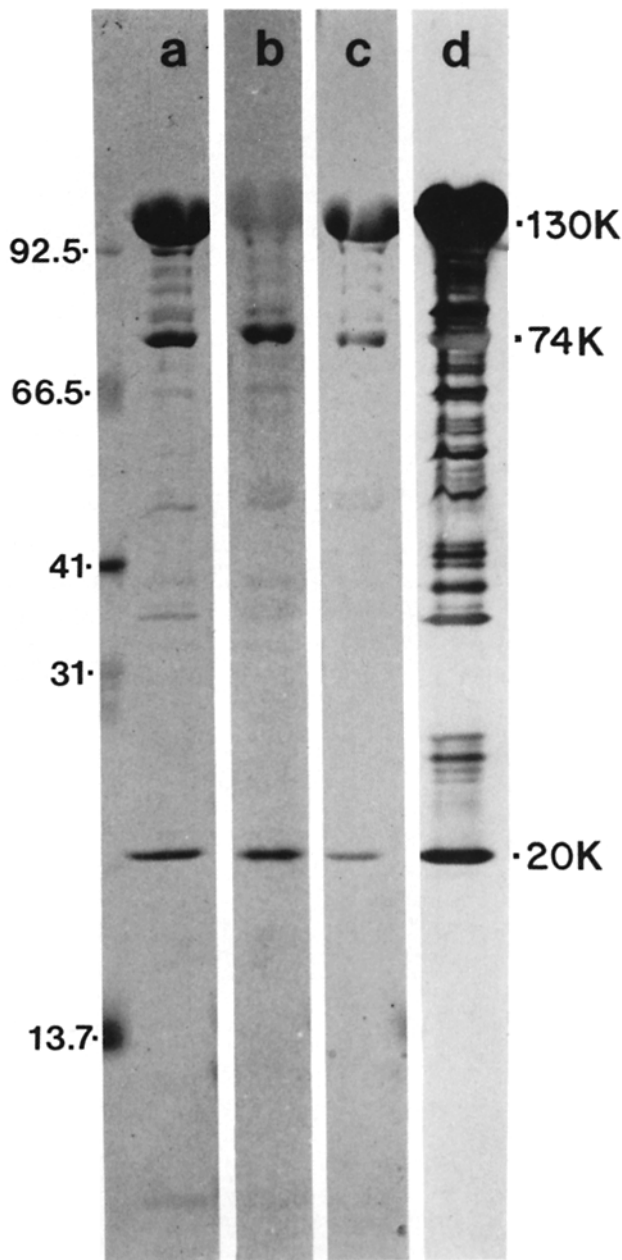


FIGURE 6 Western blot analysis of the antiarray antibody. (a) This lane demonstrates the typical protein band pattern of surface array preparations in SDS-PAGE, and is a Coomassie Blue-stained portion of the polyacrylamide gel. A lane containing molecular weight standards is partially shown at left: Phosphorylase b (92,500), bovine serum albumin (66,500), alcohol dehydrogenase (41,000), DNAase 1 (31,000), and RNAase A (13,700). The array proteins discussed in the text are identified at right. (b) Another lane of surface array proteins is shown as it appears when transferred to nitrocellulose and stained with Amido Black (29). (c) This lane shows the amount of protein that remains in the polyacrylamide gel after the electrophoretic transfer process, as visualized by Coomassie Blue staining. It is the lane used for the antibody labeling shown in d. (d) This lane represents the labeling observed when electrophoretically transferred proteins (from lane c) are treated with antiarray antibody and subsequent peroxidase-catalyzed color reaction steps. Note the much reduced labeling of the 74K polypeptide.

An important aspect of stalk growth is how the cell determines the site at which the stalk develops. The stalk forms at the precise location occupied by the flagellum and pili after they are discarded or otherwise removed during cell development. Early stages of stalk outgrowth visualized by colloidal-gold labeling indicated that the region was small and spatially restricted. The nature of such a membrane specialization or site restriction is unknown, but it appears to be related to the cell's selection of a site for division. That is, the stalk is elongated at the site of the previous division, and the similarity in the pattern of surface array formation on the developing stalk and the region of cell division is striking. The mechanism of surface array formation may be similar in both cases; indeed, such similarities suggest that some of the mechanisms used in orienting and producing cell poles during division may also be used in determining the site of stalk production later in the cell cycle.

Our studies also indicate that stalk initiation is under temporal cell cycle control. Terrana and Newton (24) demonstrated, by blocking cell division with penicillin G, that a step just before the previous division was necessary for stalks to form. However, there is no evidence that subsequent elongation of the stalk is under cell cycle control. In fact, other environmental factors such as phosphate limitation can have a dramatic effect on stalk length (17). Our experiments confirm the apparent lack of cell cycle temporal control of stalk growth after initiation.

A model can be proposed that accounts not only for the morphogenetic events occurring at the differentiating cell pole, but also for the asymmetry of differential expression of polar structures by each of the daughter cells. The fundamental premise of the model is that, superimposed on the processes by which bacterial cells orient their division planes, *Caulobacter* cell division also creates identical complexes at each new cell pole (Fig. 7). These complexes serve to organize the sequence of differentiation events progressing from the expression of flagellum, pili, and phage receptors to stalk formation. The pole actively synthesizing polar structures would be dominant and preclude activation of the new complex. Termination of the differentiation sequence with the production of a stalk would permit activation of a new cascade at the quiescent polar complex. Examination of the differentiation pattern (Fig. 7) reveals that this single activation of the differentiation sequence is sufficient to account for the asymmetry of the entire differentiation process in each of the daughter cells.

We previously suggested and provided presumptive evidence for existence of a complex that coordinates the expression of all polar structures, including the stalk (25, 26). The data presented in this paper further suggest a correlation between polar membrane development and morphogenesis. The production and persistence of distinct polar regions during surface array biogenesis as seen in this study, coupled with our earlier studies, suggest that a specialized membrane complex may exist in this region. In addition, evidence from other molecular studies (24, 26, 27) has suggested a correlation between cell division and polar development. Overall, the model offers a reasonable explanation as to how the cell coordinates the processes of differentiation and reproduction both temporally and spatially.

The results of this study also raise some other interesting questions that may relate to membrane assembly patterns in more complex systems. For example, does the pattern of surface layer synthesis, which seems intimately involved with stalk development, only reflect this differentiation process or might



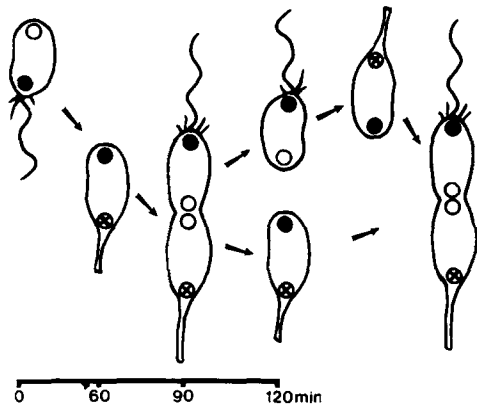


FIGURE 7 The diagram summarizes a model for the biogenesis and differential expression of polar structures in *Caulobacter* development as detailed in the text. The open circles (○) represent identical complexes created at each new cell pole as a consequence of cell division. The closed circles (●) represent an "active" polar complex, i.e. one that is engaged in the expression of flagellum, pili, and other swarmer cell characteristics. Production of the stalk signals the completion of the differentiation sequence and the pole becomes inactive (⊗). At no time does a cell possess more than one active pole. Completion of the differentiation sequence (⊗) signals the polar complex formed in the previous cycle (○) to become active (●). Thus, a developmental pole can be viewed as a complex that has its origins at division, focuses swarmer cell characteristics during its active phase, and becomes quiescent after formation of a stalk. The model also describes how the biogenesis of identical polar complexes can result in the asymmetric development that occurs in *Caulobacter*.

it have an active role in stalk formation? There is no precedent for such a role of bacterial surface layers; however, one can imagine that a repetitive structure that forms an interconnected two-dimensional network may be involved with at least the initial steps of the membrane "moulding" processes. These experiments have also demonstrated a more subtle feature of differentiation. Most studies of development in *Caulobacter* and other model systems examine structures or components whose expression is temporally controlled during the cell cycle. For *Caulobacter*, the flagellum and pili are examples. However, in the case of the surface layer, there is no indication that the composition of the surface array on the stalk is different from that on the remainder of the cell (3), nor is there evidence that the rates of synthesis of surface layer proteins are modulated during the cell cycle (26). Instead, by regulating the manner and location of surface array assembly, different areas of the cell become differentiated. How a single gene product can manifest such alternative patterns of expression is clearly an important aspect of understanding the cell differentiation process.

We acknowledge the expert technical assistance of Greg Schultz.

This work was supported by National Science Foundation Grant PCM-8021551 and National Institutes of Health Grant GM25527.

Address correspondence to Dr. Agabian.

Received for publication 16 February 1982, and in revised form 1 June 1982.

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