REQUIREMENT FOR CELLULAR ASSOCIATIONS IN THE DEVELOPMENT OF CAULOBACTER CRESCENTUS

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Summary: Caulobacter swarmer cells require the presence of the stalked cell for continued growth and development. The active component(s) involved in stimulating swarmer growth is associated with the stalked cell envelope and can be released in soluble form following repeated freeze-thawing of stalked cell fragments. The soluble cell envelope fraction contains a large protein whose presence is necessary for swarmer stimulation.

INTRODUCTION

The dimorphic bacterium, Caulobacter crescentus, carries out a defined progression of cellular events which are both spatially and temporally controlled (1). Upon division two morphologically distinct cell types are produced, the larger stalked cell and the polar-flagellated swarmer cell (1). The stalked cell initiates a new round of replication immediately following division and therefore continues the same sequence of developmental events with each cell cycle (2). No DNA synthesis has been shown to occur in the swarmer cell (2). The swarmer cell continues its cycle by synthesizing a stalk and releasing its flagella into the media (1). This irreversible process represents a developmental program whose control elements are not clearly understood. Since the stalked and swarmer cell share a common cytoplasm and it has been shown that the pattern of chromosome segregation is random (3), the differential control of DNA synthesis does not appear to be under the sole influence of soluble cytoplasmic factors or specific structural alterations at initiation sites on the chromosome. Degnen and Newton have suggested that the regulation of DNA synthesis may be affected by structural alterations at specific membrane-associated sites located in one or both cell types (2).

In view of the observations which indicate that swarmer development

can occur in the absence of DNA synthesis, we conducted a series of experiments to determine what factors are required for the initiation of swarmer growth. The results of these experiments are presented in this communication.

METHODS AND MATERIALS

Caulobacter crescentus (ATCC 15252) was grown in Peptone-Yeast extract media (PYE) as described by Pointdexter (4). Cultures were grown in a New Brunswick Environmental Shaker at 30°C and 150 r.p.m. and growth was followed using a Klett-Summerson Colorimeter (420 nm filter). Cell counts were made using a Petroff-Hauser counting chamber.

Swarmer cells were isolated from mid-log cultures using a modification of the technique described by Stove and Stanier (5). An initial 15 minute centrifugation at 1,000 x g in an IEC HN-S Swing bucket centrifuge followed by two 10 minute runs yielded swarmer populations of greater than 95% purity. Swarmers were then incubated at 30°C for 60 minutes in the culture media prior to growth studies to insure synchronous growth of the swarmers. Checks on each isolation as well as calculations on the distribution of cell types throughout synchronous growth were performed on negatively-stained cells scanned in a Philips EM200 electron microscope.

Cell fragments used in mixing studies were prepared from washed (3x) stalked cells harvested in their late-log growth phase, disrupted in a French Pressure Cell at 20,000 p.s.i. and treated with DNAse (100μg/ml) for 30 minutes at room temperature. The fragments were then collected by centrifugation for 30 minutes at 27,000 xg, the pellet fractions washed 3x in fresh PYE and stored in PYE for use in the swarmer studies.

Soluble cell envelope factor was released from stalked cell fragments suspended in 0.1M phosphate buffer (pH 7.2) following repeated freeze-thawing (5x) in an acetone-dry ice bath. The cell envelope fraction was stored at 4°C.

RESULTS AND DISCUSSION

During attempts to separate swarmer cells from stalked cells we observed that homogeneous swarmer preparations (95%) were unable to grow unless stalked cells were present (Figure 1). Following the addition of stalked cells, logarithmic growth resumed in the swarmer cell culture after a short lag period. The lack of swarmer growth in the absence of stalked cells suggests that either 1) the medium had become depleted or contained an inhibitor 2) stalked cells secrete a factor required for swarmer growth or 3) contact between stalked and swarmer cells is a prerequisite for swarmer growth. Our experiments lead us to suspect the latter possibility. We have discounted media effects since replenishment of a washed swarmer culture with fresh PYE media or separation of swarmer cells from stalked cells by a

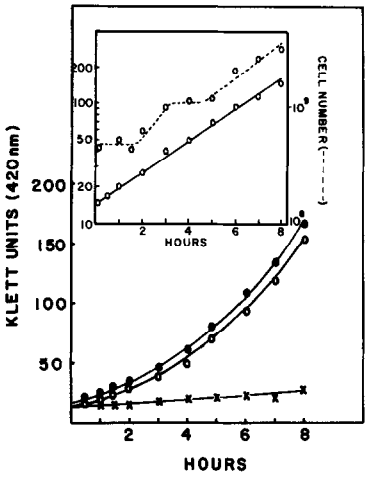

Figure 1: Growth of swarmer cells following the addition of stalked cell fragments or soluble cell factor, (O—O—O); mid-log culture centrifuged and resuspended in fresh media, (●—●—●); swarmers, no addition (X—X—X). Insert: logarithmic plot of factor-treated swarmers and cell number.

dialysis membrane did not stimulate swarmer growth in the absence of stalked cells. Similarly, secretion of an active component by stalked cells seems unlikely because there is no initiation of swarmer growth by addition of stalked cell cytoplasmic extracts or with concentrated pre-conditioned stalk cell media. However, the addition of U.V.-killed stalked cells to swarmer cells resulted in swarmer cell growth (Table 1).

The results presented in figure 1 demonstrate that treatment of swarmers with stalked cell fragments rather than whole stalked cells stimulates swarmer growth. These observations suggest that the stalked cell envelope or some component associated with the cell envelope plays a role in triggering swarmer development.

To determine whether the stimulation was specific for Caulobacter, cell envelope fragments were prepared from E. coli, P. fluorescens and B. subtilis and tested in swarmer populations. No growth was observed using these cell fragments. The addition of swarmer cell fragments also had no effect.

Table 1

Stimulation of Swarmer Cell Growth

Experiment	Growth Stimulation (% of control)
Swarmer cells (95%) - No addition	0
Swarmer cells (95%) +	
Whole stalked cells (control)	100
Stalked Cell Fragments	100
U.V. Killed Stalked Cells	90
Sonicated Stalked Cell Fragments	95
Dialyzed Stalked Cell Fragments	95
Freeze-thawed (5x) Fragments-Pellet	0
Freeze-thawed (5x) Fragments-Supernatant	100
Protease Treated Cell Envelope Factor	0

The component(s) responsible for the swarmer stimulation was released from the stalked cell fragments in soluble form by repeated freeze-thawing. Cell fragments subjected to this treatment lost all ability to stimulate the growth of swarmer populations. All stimulatory activity was now associated with the solubilized cell envelope fraction (Table 1).

Solubilized cell envelope factor stimulates the growth of swarmer cells at rates equal to those observed in normal mid-log cultures (Figure 1). The observed increase in the growth rate of swarmer cells is due to the initiation of synchronous development of stalked cells from all swarmers within 60 minutes following the addition of the envelope factor. Cell counts of treated swarmer cultures demonstrate a round of synchronous division at 180 minutes after cell envelope factor addition (Figure 1, insert).

Passage of the soluble cell envelope fraction through Sephadex G-150 or dialysis for 48 hours did not alter the stimulatory activity of the cell envelope fraction. Complete loss of stimulatory behavior however was achieved by incubation of the solubilized cell envelope factor with protease (40μg/ml) for 48 hours at 37°C. The complete protease hydrolysate was

added to swarmer populations with no resulting stimulatory effect. No loss of activity was observed in the cell factor fraction incubated at 37°C for 48 hours in the absence of protease. Protease treatment did not inhibit the growth of mid-log cultures or affect isolated swarmer cells. These results indicate that the cell envelope component is at least partially associated with a rather large protein molecule.

From these experiments we suggest that stalked-swarmer association is necessary for the continued progress of the swarmer cell through the Caulobacter cell cycle. The data indicate the existence of cellular interactions in Caulobacter which may be similar to those suggested for other procaryotic developmental systems (6). Wireman and Dworkin have suggested that cell interactions may play a key role in maintaining and guiding the movement of swarms in Myxobacteria (7). They have reported a requirement for the presence of swarmer cells for the conversion of the non-swarmer cell type during fruiting body myxospore induction and germination.

The important questions raised by our findings are what specific mechanisms operate in the regulation of swarmer cell development following association with the stalked cell. The coupling of swarmer cell interaction at cell envelope sites to changes within the cell are presently being studied with respect to the regulation of macromolecular synthesis.

REFERENCES

1. Shapiro, L., Agabian-Keshishian, N. and Bendis, I. (1971) Science, 173, 884-892.
2. Degnen, S. and Newton, A. (1972). J. Mol. Biol., 64, 671-680.
3. Osley, M. and Newton, A. (1974). J. Mol. Biol., 90, 359-370.
4. Pointdexter, J. (1964). Bacteriological Reviews, 28, 231-295.
5. Stove, J. and Stanier, R. (1962). Nature, 196, 1189-1191.
6. Ashworth, J. and Smith, J.E. (eds.), Microbial Differentiation 23rd Symposium, Society General Microbiol. 1974 Univ. Press London.
7. Wireman, J.W. and Dworkin, M. (1975) Science, 189, 516-523.