Encystment of *Azotobacter vinelandii*

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I. INTRODUCTION

Certain bacterial species possess the capability of differentiation through several morphogenetic changes which enable them to adapt to certain internal and external stimuli (Losick and Shapiro 1984). Upon induction, cells of *A. vinelandii* undergo a morphological process which leads to the production of one cyst per cell (Sadoff, 1975). The cysts are considerably resistant to desiccation, which confers a survival advantages upon the organism (Socolofsky and Wyss 1962). Like other prokaryotic differentiations encystment provides a relatively simple model of cellular differentiation. Like in other differentiating bacteria, vegetative growth can be separated from differentiation. Furthermore, the differentiation cycle can be synchronized by specific inducer.

There have been a great deal of morphological and physiological studies on this process. However, the mechanisms used to regulate cell differentiation can be clearly defined by careful genetic analysis of the process. Unfortunately, *A. vinelandii* has proven to be difficult for genetic analysis (Sadoff 1975). For example, it has been shown that a variety of metabolic mutants of *Azotobacter* species are difficult to isolate after mutagenesis with chemical mutagens or UV irradiation. Nevertheless recent advances in molecular genetics in *Azotobacter* species, especially in the nitrogen fixation research area, appear to be able to overcome this difficulty (Robinson et al. 1986; Kennedy et al. 1986).

II. REVIEW

1. *A. vinelandii* and its life cycle

A. vinelandii cell is strictly aerobic nitrogen-fixing soil microorganism with a morphology of prolate spheroid approximately 2×5 μm, and is capable of undergoing encystment, a form of a cellular differentiation whose morphogenetic aspects have been demonstrated by light and electron microscopy (Hitchins and Sadoff 1970). When cells are grown in nitrogen-free Burk (NFB) medium [chemically defined, nitrogen-free medium routinely used for cultivation of azotobacters (Wilson and Knight 1952)] containing beta-hydroxybutyrate (BHB) as a carbon source, growth is slow but complete encystment occurs in 48 hr (Lin and Sadoff 1968). Vegetative cells of *A. vinelandii* are motile by means of peritrochous flagella, and those cells undergoing division have a typical peanut shape. Upon induction of encystment by replacing glucose with BHB in the NFB medium, the cells lose their motility, become spherical, their cell walls become thickened, and the developing cysts turn optically refractile. The morphogenetic process yields a resting cell consisting of the central body (intine) and an outer coat (exine) (Hitchins and Sadoff 1970). The lipid accumulations in the central body consist of poly-BHB.

Cysts are oblate spheroids whose axes are approximately 1.5 μm and 2.0 μm, respectively, and thus they are approximately half the volume of vegetative cells (see Table 1, Hitchins and Sadoff 1970). They germinate at 30℃ in aerated NFB medium with an exogenous carbon source such as glucose, sucrose, or acetate (Loperfido and Sadoff 1973). This is a slow process lasting 6 to 8 hr in glucose-containing medium, during which

Table 1. Differences between vegetative cells and cysts.

	vegetative cells	cysts
• structure	typical Gram-negative rod	round wity thick coat
• microscopic appearance	non-refractile	refractile
• chemical composition		
calcium	low	high
poly-betahydroxybutyrate	low	high
phospholipids	high	low
• metabolism(oxygen uptake)	high	low
• macromolecular synthesis	high	low
• resistance to radiation	low	high
desication	low	high
heat	low	low

time the central body swells and occupies the intine volume. After eight hours, the growth of the cyst within the exine causes a ring-type fracture of the outer cyst coat, and the nonmotile dividing cell emerges. The cells regain motility prior to the postgermination division, thus completing the morphogenetic cycle (Loperfodo and Sadoff 1973). The life cycle of *A. vinelandii* is presented schematically in Fig. 1.

2. Cyst properties

Cysts are metabolically dormant cells which are considerably more resistant than vegetative cells to deleterious physical conditions (Fig. 2). In the laboratory they are "viable for more than 24 years upon storage in dry soil" (Moreno et al. 1986) whereas vegetative cells die rapidly, suggesting that desiccation resistance may be a survival attribute in nature. Cysts which have been stripped of their outer coat by chemical means or which have emerged from their exines during germination are not resistant to drying, and thus the resistance to desiccation appears to be related to the presence of the exine (Socolofsky and Wyss 1962). Desiccation resistance is used to distinguish between vegetative cells and cysts in a manner analogous to the use of heat resistance in counting bacterial endospores.

Cysts of *A. vinelandii* contain almost twice as much lipid and a greater variety of fatty acids

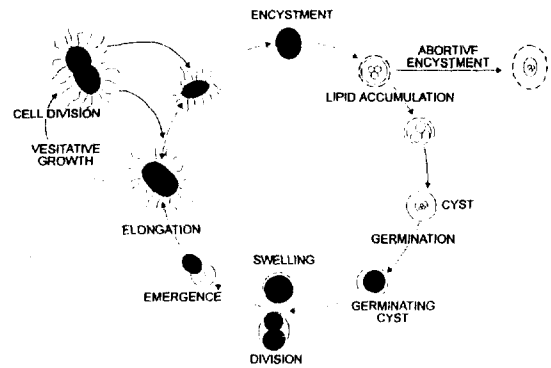

Fig. 1. Schematic diagram of the life cycle of A. *vinelandii*.

than vegetative cells (Lin and Sadoff 1969; Reusch and Sadoff 1983). When *A. vinelandii* differentiates to form cyst, the phospholipid in the membranes are replaced by novel amphiphilic lipids forming a unique membrane matrix which may contribute to the physiology and desiccation resistance of the cyst (Reusch and Sadoff 1983).

3. Induction of encystment

A. vinelandii cultures will grow on glucose, mannitol, rhamnose, and other carbohydrate, as well as on a variety of organic acids and certain alcohols (Lin and Sadoff 1969). When the cultures enter or are in late stationary phase, approximately 0.1% of the cell population encysts (Lin and Sadoff 1968). Depending on the carbon source and its concentration or other culture conditions, poly-

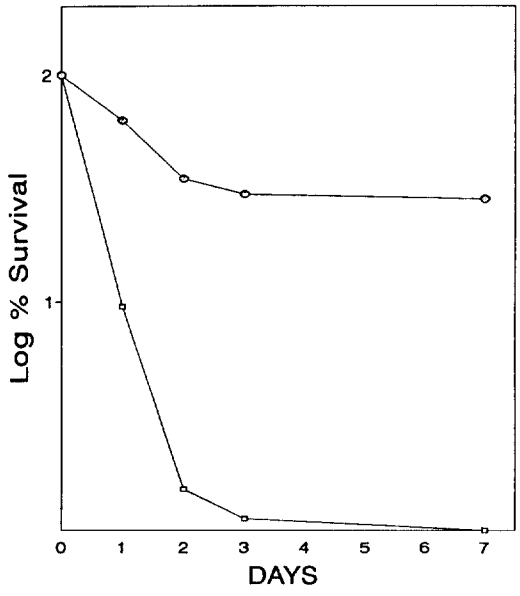

Fig. 2. Effect of desiccation on vegetative cells (■) and cysts (●) of *A. vinelandii*. The cells and cysts were collected on membrane filters and incubated at 28~30℃.

BHB accumulates in vegetative cells, and the extent of encystment in such cultures appears to be related to the intracellular levels of poly-BHB (Stevenson and Socolofsky 1966).

Specific inducers for nearly complete (more than 95% of cell population) encystment are known. These are n-butanol, beta-hydroxybutyrate (BHB) and crotonate (hydrated form of BHB) (Sadoff et al. 1971). The inducers of encystment are metabolized in contrast to an apparent nonmetabolic role for glycerol in myxospore induction (Dworkin and Gibson 1964). When BHB is added to washed, exponential phase cells in NFB medium, complete and rather synchronous encystment occurs in 48 hr (Lin and Sadoff 1968). Encystment does not occur when cells are grown on acetate despite the fact that BHB is ultimately oxidized to acetate and further to CO_2 by way of the tricarboxylic acid cycle (Sadoff et al. 1971). The specificity of the inducers of encystment (n-butanol, crotonate, or BHB) suggests that unique metabolic sequences may be involved in cyst formation. In some unknown manner, the metabolites of the inducer compound, which stop normal growth, appears to control the formation of cyst components.

Nitrogen starvation is a necessary but not sufficient condition for encystment of *A. vinelandii*; only nitrogen-fixing cells can encyst (Hitchins and Sadoff 1973). Upon induction with BHB, cells lose their nitrogenase activity within 4 hr. This decline in nitrogenase activity is accompanied by disappearance of both nitrogenase proteins from cell extracts (Kossler and Kleiner 1986).

4. Macromolecular synthesis during encystment

Immediately after induction of encystment, the rate of DNA synthesis is first sharply decreased and then stops at approximately 3 hr (Sadoff et al. 1971). The synthesis of RNA slows upon induction of encystment and stops by 12 hr. The carbon skeleton for RNA synthesis is derived from BHB (Hitchins and Sadoff 1973). Although there have been no report on whether there exist encystment specific mRNAs, it is likely that there are new mRNAs coding for the enzymes involved in the synthesis of the cyst-specific amphiphilic lipid components (Reusch and Sadoff 1983). Protein synthesis occurs throughout encystment and is the principal cellular product derived from BHB (Hitchins and Sadoff 1973). A high initial rate of synthesis continues for 9 hr postinduction and then synthesis decreases to 40% of the initial rate until cyst maturation is complete. Protein turnover has been demonstrated by using a pulse-chase procedure during exponential growth, encystment, and germination phases of the life cycle of *A. vinelandii* (Ruppen et al. 1983). It was shown that about 50% of the protein present in vegetative cells is degraded during encystment. However, the net rate of protein turnover was slightly lower than in exponentially grown cells (ca. 1% per hr). The fact that significant turnover was not observed in rifampicin-treated cells which were induced to encyst suggested that a new proteolytic enzymes might be synthesized on encystment and that the addition of rifampicin blocked synthesis of new

mRNAs (Ruppen et al. 1983).

5. Genetics of *A. vinelandii*

Although the physiology of azotobacter has been studied in great detail, a genetic analysis has been hampered by difficulties in obtaining a wide range of metabolic mutants and establishing an efficient gene transfer system. It is clear from the literature that conventional mutagenesis and selection techniques involving the use of chemical mutagens and UV irradiation have been relatively ineffective when applied to Azotobacter species. However, recent advances in molecular genetics in Azotobacter species appear sufficient to investigate the genetics of encystment. For example, transposon mutagenesis has been successfully used to isolate Nif$^-$ mutants of *A. vinelandii* with Tn5 (Kennedy et al. 1986; Joeger et al. 1986) and Fos$^-$ (inability to fix nitrogen on sugars) mutants of A. chroococcum with Tn1 (Ramos et al. 1985). In addition, two of the standard methods of gene transfer in bacterial genetics, transformation and conjugation, are now available in *Azotobacter* species (Page and Sadoff 1976; Owen and Ward 1985). Mutation generated *in vitro* was successfully introduced back into the wild-type strain to induce site-specific mutation by gene replacement (Robinson et al. 1986). Furthermore partial diploid can be obtained with which one can perform complementation and dominance tests (Kennedy et al. 1986).

III. SUMMARY

Azotobacter vinelandii is a large, aerobic, gram-negative, nitrogen-fixing bacterium. Under certain conditions the organism encysts, producing metabolically dormant cells which are analogous to endospores of the Bacillaceae in being more resistant than vegetative cells to deleterious physical and chemical agents. In turn, under favourable conditions, cysts will germinate to become active vegetative cells. Encystment, like other morphogenetic processes in prokaryote, is of interest as a relatively simple model of cellular differentiation. Recent advances in molecular genetics of *Azotobacter* species are likely to provide tools for illucidation of this interesting differentiation process.

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