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The Co-ordination of Development of Symbionts in Mutualistic Symbiosis with Reference to the Cell Cycle of the Photobiont in Lichens

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Abstract

In growing lichen thallus lobes, the photobiont cells are smaller at the growing lobe apex but become larger in the mature lobe tissues. Nuclei, as stained with DAPI and viewed with epifluorescence microscopy, were visible in 50% of the smaller cells (where not behind the central lobed chloroplast) and were in a peripheral position in the cell. In autospores within the mother cell wall, the nuclei were clustered close to the centre of the original mother cell but peripheral in the daughter cells, indicating central position of nucleus in the mother cell during mitosis. In larger "oversized" photobiont cells, the nuclei were visible in less than 50% of the cells, indicating that in many of the cells, the nucleus had already migrated to the centre and hence became obscured by the chloroplast folds. This evidence suggests that the cell division in the photobiont is regulated by an arrest of the normal cell cycle immediately prior to mitosis leading to "oversized" cells.

Keywords: symbiosis, cell cycle, lichen, development, *Diploicia canescens*, *Caloplaca flavescens*

Abbreviations: DAPI: diamidino-2-phenyl indole

1. Introduction

In every organism, growth and differentiation (development) are controlled by its genome and its environment. In symbiotic systems the development

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of each symbiont must also be coordinated so that the two symbionts in the system are fully integrated in structure and function, forming a composite organism. This process is referred to as *codevelopment* (Hill, 1985, 1989). In many symbioses, codevelopment occurs from spore (or seed) to spore (or seed) and from egg to egg. If it breaks down, the symbiosis breaks up and the symbionts grow independently, developing into separately behaving organisms.

There is a markedly wide range of environmental conditions over which two symbionts show competent codevelopment, but breakdown, or non-re-establishment, can be induced in some symbiotic organisms by environmental means. For example, legumes do not form, or form fewer, nodules under certain soil conditions (Smith and Douglas, 1987) nor do many mycorrhizas form in soils of high fertility (Smith and Douglas, 1987). *Convoluta roscoffensis* can lose its phycobionts under high carbon dioxide treatment (Boyle and Smith, 1975). *Azolla* can lose its cyanobiont under suitable growth conditions (Hill, 1987; Braun-Howland and Nierzwicki-Bauer, 1990). Different combinations of symbiont strains may have competency over different environmental ranges.

In lichens there appear to be regulatory mechanisms which maintain an algal layer of uniform thickness (Honegger, 1987; Hill, 1985) essential for the growth, development and function of the thallus. Photobiont cell division appears to be closely associated with mycobiont hyphal growth, with the hyphae separating the photobiont cells during thallus growth (Honegger, 1987; Armaleo, 1991; Hill, 1992). Hill (1989) suggested that the smaller size of photobiont cells at the apex of lobes of lichen thalli was indicative of cells undergoing rapid cell division (Phase 1 cells), since the range observed in size in the cell population coincided with the range of sizes from recently released autospores to mother cells in the process of dividing (Fig. 1). In older tissues, cell size of the photobiont was greater than that of the spore mother cells in the process of dividing, suggesting that the cells were being prevented from dividing, although large enough to do so and hence forming 'outsized' cells (Phase 2 cells) (Hill, 1985, 1989; Fiechter, 1990). These contrast with cells of cultured microorganims and microalgae which are smaller when non-dividing or slowly dividing than when rapidly growing and dividing (Raven, 1986; Donan and John, 1984;; Fantes and Nurse, 1981; Donachie, 1981).

The aim of the research reported here is to investigate the regulation of the symbiont cell cycle, an important aspect of symbiotic codevelopment. In the lichen-photobiont *Trebouxia*, the central chloroplast is flanked by the nucleus which normally occupies a peripheral position. During cell division, the chloroplast divides first, the nucleus apparently takes up a central location (Ahmadjian, 1967) and then divides n times (where $n = lnx/ln2 - 1$ and x is the number of autospores forming within the mother cell (sporangium). The

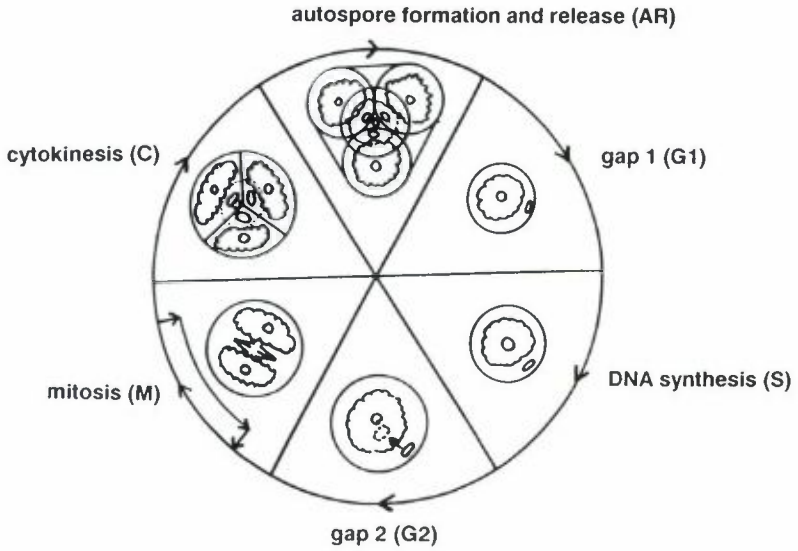

Figure 1. The Cell Cycle in *Trebouxia*.
N.B. The position of the nucleus in S and G2 and the timing of its migration to a more central position require confirmation.

nuclei of the autospores, which are then formed, would be at positions close to the centre of the original mother cell and would therefore be peripheral once more in the daughter cells. Therefore, observation of the nucleus in Phase 2 cells could help in finding out at which stage the cell cycle is arrested. This was done by investigating the position and appearance of the photobiont nucleus in growing and non-growing (mature) parts of the thallus.

2. Materials and Methods

Two lichen species were used, *Diploicia canescens* (Dickson)Massal. and *Caloplaca flavescens* (Huds.)Laundon, which were collected in a dry state from walls in Abbott's Leigh, near Bristol. These species were chosen since they both contain *Trebouxia* as the photobiont but have contrasting growth rates. *D. canescens* grows about 0.5–1.5 mm per year (depending on thallus size (Hill, 1981) while, at the same site and the same two-year period, the larger and hence most rapidly growing thalli of *C. flavescens* were found to grow only 0.39±0.03 mm per year (mean±S.E., n=46). Portions of the algal layer were dissected out, using a fine scalpel and mounted in dilute solution of DAPI between a cover slip and a slide. The coverslip was gently pressed against the slide with a slight circular motion to spread out the photobiont cells. Excess DAPI solution was blotted and the coverslip sealed with clear nail varnish to

prevent the solution drying out. Because the concentration required appeared to differ among the various species used, the strength of the DAPI solution was determined by progressively adding DAPI to a stock solution until the optimum fluorescence was obtained.

Samples of algae were removed from the apical region of the lobe within 1 mm of the lobe end and from the mature region 5 mm from the lobe end. The diameters of over 100 photobiont cells per sample were measured to the nearest 1 μm and the cells recorded for DNA-DAPI epifluorescence in the nucleus and, if fluorescing, brightness also scored (scoring: 1 = low, 2 = medium and 3 = high fluorescence). In a few cells (< 5% of total) it was uncertain as to whether the nucleus was visible or not; these were omitted from the data.

3. Results

Position of the nucleus

In *D. canescens* and *C. flavescens* and in *Xanthoria parietina* (L.)Th.Fr., the nucleus was found at the cell periphery. After autospore formation, the nuclei were peripheral in the autospores but clustered together centrally in the sporangium, indicating the central position of the nucleus when it divided in the mother cell. These observations confirm the illustrations of Ahmadjian (1967) (Fig. 1).

Appearance of the nucleus

A stained nucleus could be seen in only about 50% of the photobiont cells of *D. canescens*. Only just over 50% of the cells would be expected to have the nucleus in front of, or along side, the central chloroplast, which almost fills the rest of the cell volume. The chloroplast, which itself epifluoresces red, obscures the nucleus from the ultra-violet light source in the other cells which, by chance of orientation, have the nucleus behind the chloroplast. However, the proportion of cells with a visible nucleus was smaller than 50% in the larger cells than in the smaller cells. This was specially evident in the sample from the mature region of the thallus (Table 1).

In *C. flavescens*, the visibility of the nucleus was unrelated to cell size at the apex, with about 60% of the nuclei visible (Fig. 2). In the mature part of the lobe, the nucleus was only visible in 17% of the cells. No nucleus could be seen in the majority of the larger cells which were considerably larger than the photobiont cells at the lobe apex and 'outsized' (Hill, 1989).

Table 1. The visility of nuclei and cell size in photobiont of *Diploicia canescens*.

Sample position and visibility of nucleus	Cell diameter (μm)													
	4	5	6	7	8	9	10	11	12	13	14	15	16	17
(A) Tip of lobe														
(1) Visible	1.0*	2.9	7.8	10.8	3.9	2.9	10.8	1.0	0	1.0	0	0	0	0
(2) Not visible	0	0	4.9	6.9	9.8	4.9	10.8	8.8	5.9	1.0	2.0	0	0	0
(B) 5 mm from lobe end														
(1) Visible	0	0	0	1.0	4.8	9.6	17.3	15.4	3.8	1.9	2.9	1.0	1.0	0
(2) Not visible	0	0	0	0	1.0	2.9	6.7	1.9	12.5	6.7	3.8	4.8	0	1.0

(*numbers are percentage of photobiont cells in each sample)

Table 2. Intensity of fluorescent staining of nucleus

Photobiont sample	Cell diameter (μm)													
	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Diploicia canescens														
Tip of lobe	3*	2.7	2.5	1.7	1.5	3.0	2.3	1.0	0	1.0	0	0	0	0
5 mm from lobe end	0	0	0	3.0	2.0	2.6	2.4	2.1	2.3	2.5	1.3	2	1	0
Caloplaca flavescens														
Tip of lobe	0	0	2.3	1.7	1.7	1.0	0.9	0	3.0	1.0	0	2.0	0	0
5 mm from lobe end	0	0	0	0	1.0	2.0	2.8	1.3	1.3	1.5	1.0	1	3	1

(*numbers are mean scores of intensity of fluorescence)

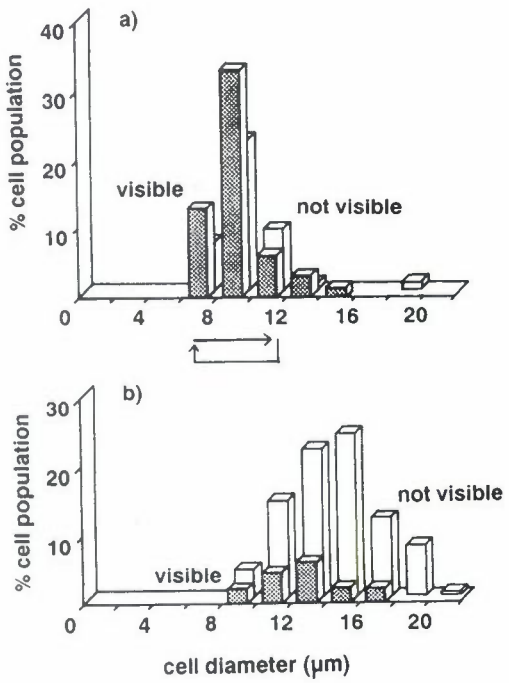

Figure 2. The proportion of cells with visible nuclei in the photobiont of *Caloplaca flavescens* (stained with DAPI) as a function of cell size. (a) tip of the lobe; (b) 5 mm from lobe end. The arrows under the upper histogram indicate the probable size changes due to the cell cycle based on 4 autospores per mother cell (see Fig. 1).

Loss of visibility could be due to progressive loss of DNA or DNA-DAPI staining in the larger (i.e. older) cells rather than to masking by the chloroplast. However, the mean scores for the brightness of the epifluorescence were not linked to cell size (Table 2) and hence the cell cycle, suggesting that this was not the case.

4. Discussion

The cell cycle in the photobiont may be represented diagrammatically as has been done for other symbionts (Hill, 1989) (Fig. 3). Dividing cells at the apex are represented by the circle (Phase 1 cells) and non dividing 'oversized' cells by the horizontal line (Phase 2 cells). At which stage of the cell cycle are Phase 1 cells arrested to produced Phase 2 cells? The lack of visibility of the nucleus found in the larger Phase 2 cells suggests that the nucleus may have assumed a more central position in the cell where it is obscured by the

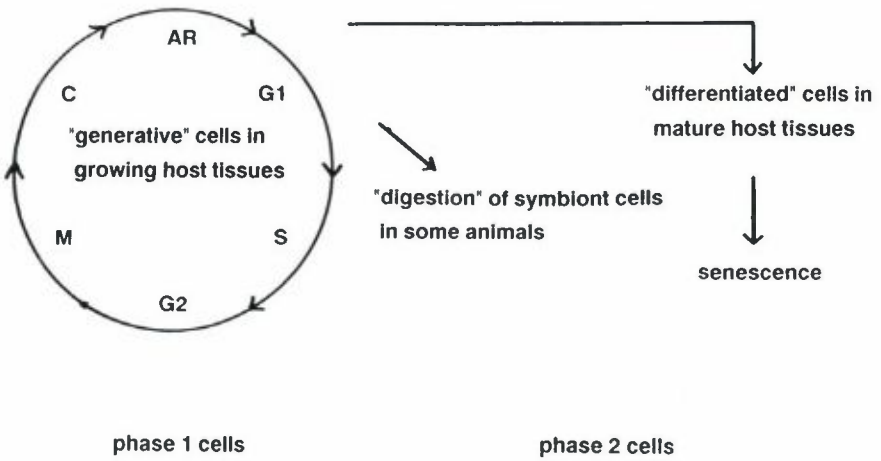

Figure 3. General scheme for the cell cycle in microbial symbionts.

chloroplast. It would be interesting to know whether in *Trebouxia*, migration of the nucleus occurs, as one might expect, after, rather than before S phase. If so, then arrest of the cell cycle would appear to be at G2. It is the initiation of DNA synthesis (S) which sets off the start of a new turn of the cell cycle and in most organisms, non-dividing cells are arrested in G1 rather than G2 (Fantes and Nurse, 1981).

In a growing lobe, horizontal expansion of the tissues occurs most at the distal end and declines gradually to zero towards the lobe base (Hill, 1981). If the cell cycle of the photobiont were not arrested, there would be a build-up of cells in the older parts but the regulation of the photobiont cell cycle keeps the number of photobiont cells constant. In more rapidly growing lobes which are wider (Hill, 1992), the photobiont cells may pass through more cycles before arrest than in narrower slower-growing lobes.

The arrest of the cell cycle in Phase 2 cells occurs in the symbionts of other symbioses such as *Azolla*, leaf nodules and legume root nodules, although it is not generally known how this occurs. An understanding of this might assist in elucidating the mechanism(s) of regulation of symbiont numbers. In gut symbionts, phase 1 cells are digested further down the gut and limited regulation may occur. But in insect bacterial symbiotes, for example, in the mycetocytes of aphids, regulation of numbers in the developing nymph is clearly likely, although Phase 2 cells do not occur in the adult in which the symbiotes degenerate (L. Whitehead and A. Douglas, pers. commun.). In *Hydra* McAuley (McAuley, 1981, 1990, McAuley and Darrah, 1990) has

demonstrated how *Chlorella* cell numbers per host cell are regulated by the coordination of cell division in the symbiont and the host. The host could regulate *Chlorella* division by availability of nitrogen (McAuley, 1991), and indeed symbiotic *Chlorella* does have low levels of amino acids (McAuley, 1987), but Rees (1989) has found that freshly isolated *Chlorella* had a pattern of reduced ammonium assimilation unlike nitrogen-starved cells.

Low pH stimulates maltose release and reduces growth rate proportionately (Douglas and Smith, 1984), and McAuley (1992) has found that *Chlorella* cultured at low pH had amino acid pools, C:N ratios, chlorophyll and nitrogen contents similar to those of symbiotic cells rather than those grown in nitrogen-limited culture. Hill (1989) investigated the incorporation of $^{14}CO_2$ into samples from increasing distance from the lobe end in *Peltigera*. With increasing distance from the apex, there was a substantial increase in amount of ^{14}C fixed, although the number of photobiont cells was not found to increase (Hill, 1985). The amount of carbon released by the photobiont more than doubled while use of carbon for macromolecular synthesis by the photobiont declined. These features also suggest the possibility of the photobiont being carbon-limited by loss to the fungus. Meindl and Loos (1990) have found that in isolated photobiont (and free-living *Nostoc punctiforme*) in the presence of FCCP (carbonyl cyanide p-trifluoromethoxyphenylhydrazone), cellular release of soluble carbohydrate (glucose) was a passive and not an active process and apparently occurred by the action of an amylase activity on endogenous B1,4-glucan (glycogen). Whether this is a general characteristic of Phase 2 photobiont cells in lichens requires further investigation.

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