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Nucleic acid synthesis accompanying the recovery of cell division and chloroplast development in "giant" cells of the Emerson strain of *Chlorella*

Luong Van Thinh and Dilwyn J. Griffiths

Botany Department, La Trobe University, Bundoora, Vic. 3083, Australia

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The light-induced recovery of cell division and chloroplast development in "giant", "bleached", cells of the Emerson strain of *Chlorella* takes place without any increase in DNA and is relatively insensitive to mitomycin C and 5-bromouracil. 5-Fluorouracil inhibits cell division only when it is supplied during the early stages of recovery, perhaps by interfering with that phase of RNA synthesis which occurs during the first 6 hr of recovery. This early burst of RNA synthesis is more sensitive to chloramphenicol than is the second phase of RNA synthesis, suggesting that a significant proportion of it may originate in the chloroplast. Evidence is presented which suggests that 5-fluorouracil interferes with chloroplast development primarily through an effect on chlorophyll synthesis. The possible significance of these observations in relation to nuclear-chloroplastic interractions is discussed.

The various systems that have been used in studies of the re-greening of "bleached" algal cells show varying degrees of interdependence between cell division and chloroplast development. At one extreme is the situation represented by "bleached" *Euglena* cells which divide as they are re-greening and which have to be provided with a special medium to prevent cell division (*1*). This suggests that although under normal circumstances (in the light on a complete medium), the division of *Euglena* chloroplasts is linked to cell division in such a way as to maintain the chloroplast number more or less constant from generation to generation, the link is a fairly loose one. Most other systems show a greater degree of interdependence between the two processes. Thus, during the re-greening of a dark-grown mutant of *Chlamydomonas* (*2*), there is no cell division until two to four hr after completion of chloroplast development. A similar situation is encountered in the re-greening of "glucose-bleached" cells of *Chlorella protothecoides* where cell division is delayed until after the 24th hr of recovery, when the chloroplast has reached an advanced stage of recovery (*3*). It is clear, therefore, that in *Chlamydomonas* and in *Chlorella protothecoides*, the pace of nuclear division is more firmly geared to that of plastid development than is the case in *Euglena*. However, with the *Chlorella protothecoides* system, at least, it is possible, by various treatments, to bring about a separation of cell division and chloroplast development (*4*, *5*); indeed, the so-called "etiolated" cells of this alga represent a system in which re-greening takes place in the absence of cell division (*3*).

In this paper, the general question of the linkage between cell division and

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chloroplast development is further examined using a system based on the recovery of "giant", "bleached" cells of the Emerson strain of *Chlorella*. The "giant" cells are produced when synchronous cultures are maintained under carefully-controlled heterotrophic conditions (*6*). They result from a failure of cell division and are characterized by their abnormally large size and by their severely degraded chloroplasts (*7*). When the "giant" cells are returned to autotrophic conditions, there is a simultaneous recovery of cell division and chloroplast development (*8*, *9*), although, by the use of certain antibiotics (particularly chloramphenicol), the two processes can be separated (*10*). The "giant" cells therefore represent a system in which both cell division and chloroplast development are temporarily held in check, and their light-induced recovery is clearly particularly suitable for studies of the interrelationships between nuclear and chloroplastic development. The work to be described here explores this problem through an investigation of the nucleic acid metabolism associated with the recovery of "giant" cells. This is attempted firstly, by testing the effect upon recovery of certain substances known to interfere with various aspects of nucleic acid metabolism and secondly, by analysis of changes in DNA and RNA levels during the course of recovery.

Materials and methods

The organism used in this investigation is a strain of *Chlorella* originally isolated by Emerson and designated in the Cambridge collection of algae and protozoa as 211/11 n. The strain was formerly named *C. vulgaris*, Beijerinck 211/11 n, Emerson and listed as being possibly the same as *C. pyrenoidosa*, Chick, Emerson No. 3 (211/8 h). In view of this confusion, the specific name has now been disregarded and the organism referred to as the Emerson strain of *Chlorella*.

A homogeneous population of "giant" cells was prepared as previously described (*11*). Recovery of the "giant" cells following their return to autotrophic conditions was monitored in terms of cell growth and cell division (Coulter counter, Model B), pigment synthesis (spectrophotometric estimation from acetone extracts), and photosynthetic capacity (in terms of oxygen evolution at saturating light intensity, measured at 25°C by means of an oxygen electrode). Changes in DNA and RNA levels during recovery were estimated by the method of Schmidt and Thannhauser (*12*) as modified by Aoki and Hase (*3*) and the results expressed as optical density at 260 nm (OD_{260}), corrected for absorbance at 320 nm. In general the various parameters are expressed per ml of culture, in order to give a clear indication of the overall increase of that particular component during recovery.

Results

The effect of mitomycin C and 5-bromouracil upon recovery

The levels of recovery achieved at the end of a 24 hr period in the presence of various concentrations of mitomycin C and 5-bromouracil, respectively, are shwon in Fig. 1a and b. Mitomycin C is an antibiotic known to suppress DNA synthesis in *Chlorella* (*4*) whilst 5-bromouracil is a thymine analogue which interferes with DNA replication in various micro-organisms and which has been shown to become incorporated into the DNA of *Chlorella* (*13*).

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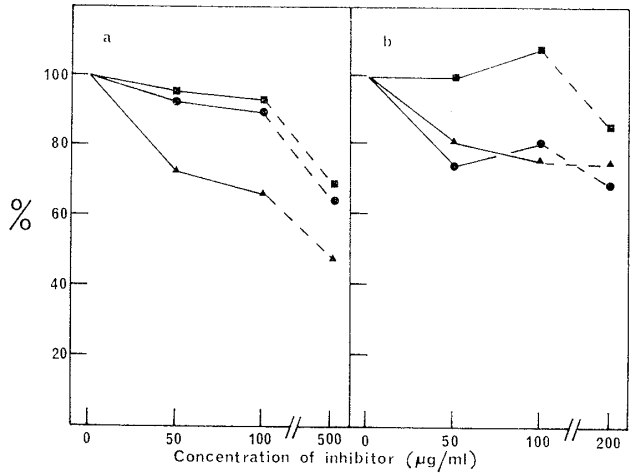

Fig. 1. *The population density* (-●-), *chlorophyll content* (-■-) *and capacity for photosynthetic oxygen evolution* (-▲-) *of cultures subjected to a 24 hr period of light recovery in the presence of various concentrations of*:-(*a*)-*mitomycin C*; (*b*)-*5-bromouracil*. The results are expressed as a percentage of the corresponding control values.

It can be seen that in order to achieve a 50% inhibition of the photosynthetic capacity, and a 30% inhibition of chlorophyll synthesis and cell division, mitomycin C must be present at a concentration of 500 μg/ml. This is well in excess of the concentration previously shown to completely inhibit cell division during the re-greening of glucose-bleached cells of *Chlorella protothecoides* (*4*). Moreover, since at this highest concentration, the mitomycin C solution is slightly coloured, it is possible that some of the observed inhibitory effect may be attributed to an interference with the availability and quality of light. However, there is no doubt that the development of a photosynthetic capacity is the aspect of recovery which is most sensitive to mitomycin C, with cell division as the least sensitive.

None of the concentrations of 5-bromouracil tested produced any marked effect on any aspect of recovery, even though the concentrations used are vastly in excess of those which have been previously shown to interfere with the DNA metabolism of various microorganisms (*14*, *15*).

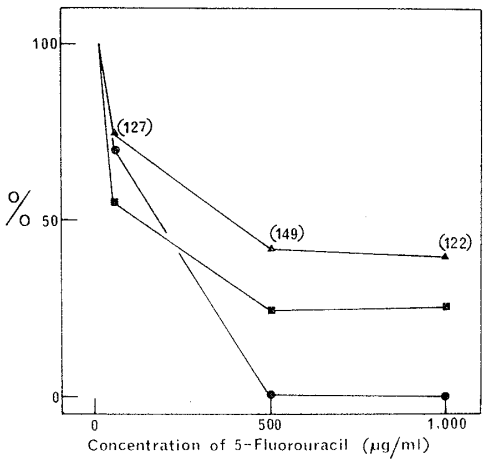

Fig. 2. *The population density* (-●-), *chlorophyll content* (-■-) *and capacity for photosynthetic oxygen evolution* (-▲-) *of cultures subjected to a 24 hr period of light recovery in the presence of various concentrations of 5-fluorouracil*. The results are expressed as a percentage of the control values. The figures in brackets adjacent to the triangles denote the rate of photosynthetic oxygen evolution per mg of chlorophyll, expressed as a percentage of the corresponding control value.

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The effect of 5-fluorouracil upon recovery

5-Fluorouracil (5Fu), an analogue of uracil, affects different aspects of the light recovery of "giant" cells to different extents (Fig. 2). Thus, a concentration of 500 μg/ml completely inhibits cell division but has a significantly less drastic effect upon chlorophyll synthesis and the development of a photosynthetic capacity. When photosynthetic oxygen production is expressed as a function of the chlorophyll content, the values obtained with all concentrations of 5Fu are consistently higher than the control values (Fig. 2). Clearly, the primary effect of 5Fu, as far as photosynthesis is concerned, is upon chlorophyll synthesis rather than upon the synthesis of photosynthetic enzymes.

Fig. 3 shows the effect of supplying 5Fu (500 μg/ml) to cultures at different

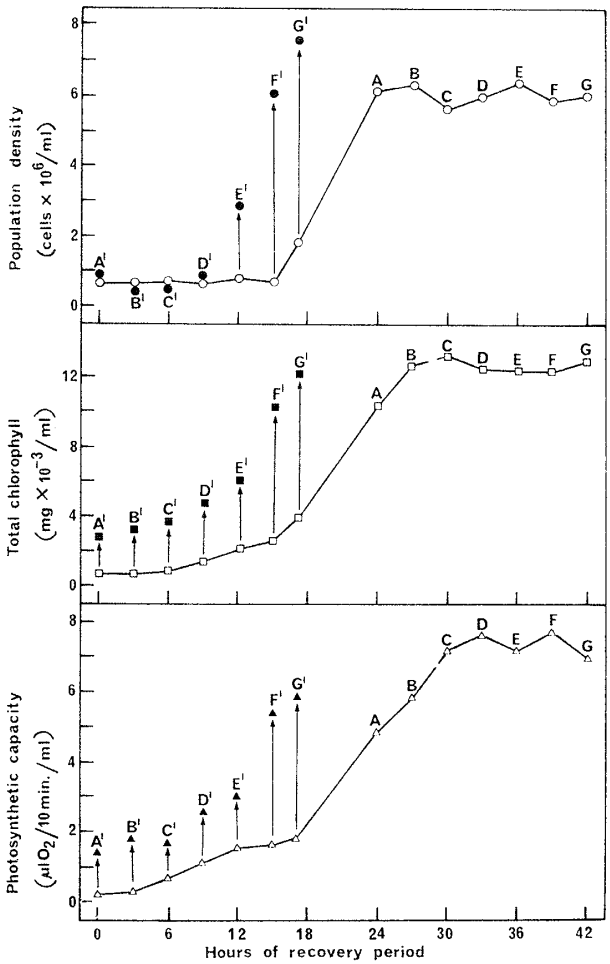

Fig. 3. *The effect of 5-fluorouracil (500 μg/ml) supplied at different stages of the light recovery period upon cell division, chlorophyll synthesis and the development of a photosynthetic capacity.* The development of these three aspects of the recovery process in control cultures is shown by the open circles, squares and triangles, respectively. The time of addition of the inhibitor is indicated by the arrows and the closed circles, squares and triangles at the point of each arrow, show the respective values after a 24 hr exposure to the inhibitor. The control corresponding to each treatment can be located by pairing the letters (eg. A′, 24 hr exposure to 5-fluorouracil starting at the beginning of the recovery period; A, corresponding untreated, control culture).

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stages of the recovery period. The samples were taken 24 hr after application of the analogue and various aspects of recovery compared with that which had occurred in corresponding control cultures. Application of 5Fu during the first 15 hr of recovery strongly suppresses autospore production. However, if treatment with the analogue is delayed until after the 15th hr of recovery, the level of autospore production is unaffected. Chlorophyll synthesis and the development of a photosynthetic capacity are both affected in a similar way except that, with these parameters, early application of the analogue was slightly less disruptive. Expressing photosynthetic oxygen production as a function of chlorophyll content again gave values suggesting that the major effect of the analogue on photosynthesis is through its effect on chlorophyll synthesis. The effect of 5Fu on carotenoid synthesis follows the same pattern as its effect on chlorophyll synthesis.

The effect of 5Fu on recovery could not be reversed by the simultaneous application of uracil, even when the uracil was supplied at a concentration exceeding that of its analogue by a factor of ten. This suggests that the disappearance of the suppressive effects of 5Fu when applied late in the recovery period (after 15 hr), probably cannot be explained by assuming that uracil (or its derivative) is formed in the cells as this time in sufficiently high concentrations to alleviate completely the effects of 5Fu (*5*).

Changes in DNA and RNA content during the course of recovery

In "giant" cells, the optical density value corresponding to DNA is almost identical with that corresponding to RNA (Fig. 4). This is quite different from the situation (not described fully in this paper) in synchronous autotrophically-grown

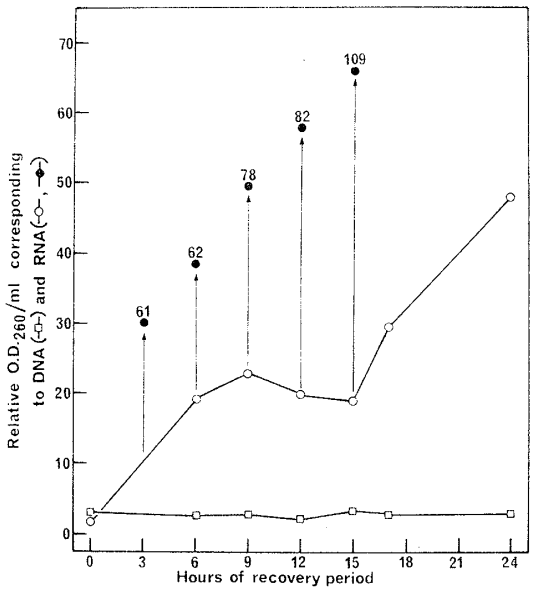

Fig. 4. *Changes in total DNA* (-□-) *and RNA* (-○-) *during a 24 hr period of light recovery.* The closed circles (-●-) show the RNA levels after a 24 hr period of exposure to chloramphenicol (100 μg/ml) supplied at the time indicated by the arrows. The figures adjacent to the closed circles show the RNA levels of the chloramphenicol-treated cultures as a percentage of the corresponding control value.

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cultures where, during the early stages of the growth phase, the RNA value exceeds the DNA value by a factor of 6 to 7. Comparison of the values corresponding to RNA on a cellular basis shows that the "giant" cells are much lower in RNA than the autospores from which they are derived. Clearly, the seven day period of heterotrophic growth has been accompanied by a significant degradation of RNA.

During the 24 hr period of recovery following transfer of the "giant" cells to autotrophic conditions, there is an appreciable increase in the absorbance value corresponding to RNA, whilst, during the same period, the value corresponding to DNA remains virtually constant (Fig. 4). RNA synthesis during light recovery takes place in two distinct phases, an early phase marked by an eight to nine-fold increase in absorbance value and a later phase coinciding with autospore production (compare with Fig. 3). It is interesting to note that by the end of the initial burst of RNA synthesis, the ratio of RNA/DNA has been restored to a value (approximately 7) which is typical of autotrophically-grown cells in the early stages of their growth phase.

Chloramphenicol, an antibiotic previously shown to selectively inhibit chloroplastic protein synthesis in our system (*10*), has a markedly inhibitory effect on the early burst of RNA synthesis. Thus, if chloramphenicol (at a concentration of 100 μg/ml) is added during the early stages of recovery, the total RNA production is reduced by approximately 40 per cent (Fig. 4). If addition of the antibiotic is delayed until the 12th to 15th hr of recovery, there is almost no reduction in the level of RNA finally attained. Clearly the second burst of RNA synthesis which coincides with autospore production, is not affected by chloramphenicol.

Discussion

In the re-greening system described in this paper, the production of green, photosynthetically active autospores from the "bleached", "giant", cells, takes place without any increase in DNA. Clearly, the "giant" cells contain DNA levels adequate for autospore production and it is hardly surprising, therefore, that recovery is relatively insensitive to those substances known to interfere with DNA replication. In this respect, our system differs from the *C. protothecoides* system used by Aoki and Hase (*4*), where a concentration of mitomycin C (200 μg/ml) which is without effect on the re-greening of glucose-bleached cells, nevertheless suppresses cell division completely. This was interpreted by them as indicating that whilst cell division was dependent upon the synthesis of new DNA, greening was controlled by chloroplastic DNA which was already present in the glucose-bleached cells. In our system, all aspects of recovery appear to be virtually independent of the synthesis of new DNA.

Under heterotrophic conditions, autospore production is obviously held in check at a step subsequent to DNA replication but which is overcome when the "giant" cells are returned to autotrophic conditions. The suppression of autospore production by 5Fu presumably reflects an effect of that analogue upon RNA metabolism. It is interesting to note that if treatment with 5Fu is delayed until after the first 12 hr of recovery, its inhibitory effect on cell division completely disappears. Clearly, some preparatory steps for cell division, sensitive to 5Fu, take place during the early hours of exposure to light. The initial burst of RNA synthesis which occurs during the first 6 hr of recovery is perhaps an important

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component of this preparatory phase. In the greening of dark-grown cells of *Euglena*, 5Fu has been shown to interfere specifically with ribosome production and to be ineffective when supplied after ribosome production has been completed (*16*). According to ultrastructural evidence previously published (*8*), the first 6 hr period of recovery in our system is also the time of most rapid ribosome formation, particularly within the chloroplast. The sensitivity to chloramphenicol of the early burst of RNA synthesis is a further indication that it may have a significant component of chloroplastic origin (perhaps as ribosomal RNA).

When 5Fu is supplied at the start of the recovery period, it affects cell division more drastically than it does chloroplast development. Perhaps some of the RNA associated with chloroplast development is already partially present at the start of the recovery period, presumably represented by the rather low optical densities recorded for this fraction at that time. When the photosynthetic capacity of 5Fu-treated cultures is expressed as a function of the chlorophyll concentration, it is clear that the major effect of the analogue is upon chlorophyll synthesis. It has been shown that in our system (*10*), as in various other re-greening systems (*17*, *18*), chlorophyll synthesis is partially dependent upon protein synthesis centered on the cytoplasmic ribosomes. This suggests either that the RNA associated with the synthesis of photosynthetic enzymes is immune to 5Fu or, more probably, that it is already partially present in the "giant" cell chloroplast at the start of recovery. The latter interpretation would certainly be in line with results based on the re-greening of dark-grown cells of *Euglena* (*16*) where it has been suggested that while the ribosomal RNA essential for cell greening is formed during the first 19 hr of exposure to light (and is therefore sensitive to 5Fu), that associated with the synthesis of cytochrome 552 is less sensitive to 5Fu and is presumably already present before re-greening starts.

In conclusion, it can be said that one of the most interesting features of our re-greening system is that it involves chloroplast development and cell division but without any net DNA synthesis. Whilst there are other re-greening systems, such as that using "eitolated" cells of *C. protothecoides* (*4*), which do not involve DNA synthesis, they differ from ours in that they are non-dividing systems. This feature of our recovery system allows us to explain an inhibition of cell division (as occurs following early application of 5Fu) as some effect other than an interference with DNA replication (in the case of 5Fu, presumably through an effect on ribosomal RNA synthesis). The evidence presented here that a significant proportion of the early burst of RNA synthesis, which is clearly essential for autospore production, may be associated with the chloroplast, could perhaps be interpreted in terms of a possible control of nuclear division by the chloroplast. It must be emphasized, however, that in view of the possible non-specific effects of such a high concentration of chloramphenicol (100 μg/ml) as was used here, and of the known immunity of cell division to lower concentrations of the antibiotic (*10*), this suggestion can be no more than a very tentative one at this stage.

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References

(*1*) Stern, A. I., J. A. Schiff and H. T. Epstein: Studies of chloroplast development in *Euglena* V. Pigment biosynthesis, photosynthetic oxygen evolution and carbon dioxide fixation during chloroplast development. *Plant Physiol.* 39: 220–226 (1964).

(*2*) Ohad, I., P. Siekevitz and G. E. Palade: Biogenesis of chloroplast membranes II. Plastid differentiation during greening of a dark-grown algal mutant (*Chlamydomonas reinhardi*). *J. Cell Biol.* 35: 553–584 (1967).

(*3*) Aoki, S. and E. Hase: De- and re-generation of chloroplasts in the cells of *Chlorella protothecoides* I. Synthesis of nucleic acids and protein in relation to the process of regeneration of chloroplast. *Plant & Cell Physiol.* 5: 473–484 (1964).

(*4*) Aoki, S. and E. Hase: De- and re-generation of chloroplasts in the cells of *Chlorella protothecoides* III. Effects of mitomycin C on the process of greening and division of "glucose-bleached" algal cells. *ibid.* 6: 347–354 (1965).

(*5*) Aoki, S., J. K. Matsubara and E. Hase: De- and re-generation of chloroplasts in the cells of *Chlorella protothecoides* IV. Effects of 5-fluorouracil, dihydrostreptomycin, chloramphenicol and acridine orange on the processes of greening and division of "glucose-bleached" algal cells. *ibid.* 6: 475–485 (1965).

(*6*) Griffiths, D. J.: The growth of synchronous cultures of the Emerson strain of *Chlorella vulgaris* under heterotrophic conditions. *Arch. Mikrobiol.* 71: 60–66 (1970).

(*7*) Griffiths, D. A. and D. J. Griffiths: The fine structure of autotrophic and heterotrophic cells of *Chlorella vulgaris* (Emerson strain). *Plant & Cell Physiol.* 10: 11–19 (1969).

(*8*) Thinh, L. V. and D. J. Griffiths: Ultrastructural changes accompanying the recovery of "giant" cells of the Emerson strain of *Chlorella* I. Autospore production. *Arch. Mikrobiol.* 87: 47–60 (1972).

(*9*) Thinh, L. V. and D. J. Griffiths: Ultrastructural changes accompanying the recovery of "giant" cells of the Emerson strain of *Chlorella* II. Chloroplast development. *ibid.* 87: 61–76 (1972).

(*10*) Thinh, L. V. and D. J. Griffiths: The contrasting effects of chloramphenicol and cycloheximide on the recovery of cell division and chlorophyll synthesis in "giant" cells of *Chlorella vulgaris* (Emerson strain). *Plant & Cell Physiol.* 12: 171–179 (1971).

(*11*) Thinh, L. V. and D. J. Griffiths: Physiological changes accompanying the recovery of cell division in "giant" cells of *Chlorella vulgaris* (Emerson strain). *ibid.* 11: 621–629 (1970).

(*12*) Schmidt, G. and S. J. Thannhauser: A method for the determination of deoxyribonucleic acid, ribonucleic acid and phosphoproteins in animal tissues. *J. Biol. Chem.* 161: 83–89 (1945).

(*13*) Iwamura, T. and N. Muto: Incorporation of 5-bromouracil into the two kinds of DNA in *Chlorella*. *Plant & Cell Physiol.* 5: 359–360 (1964).

(*14*) Lion, M. B.: Search for a mechanism for the increased sensitivity of 5-bromouracil-substituted DNA to UV light II. Single-strand breaks in the DNA of irradiated 5-bromouracil-substituted T_3 coliphage. *Biochim. Biophys. Acta* 209: 24–33 (1970).

(*15*) Nagley, P. and R. G. Wake: Effect of 5-bromouracil on the pattern of deoxyribonucleic acid replication in germinating *Bacillus subtilis* spores. *J. Mol. Biol.* 43: 619–630 (1969).

(*16*) Smillie, R. M.: Formation and function of soluble proteins in chloroplasts. *Can. J. Bot.* 41: 123–154 (1963).

(*17*) Kirk, J. T. O. and R. L. Allen: Dependence of chloroplast pigment synthesis on protein synthesis: Effect of actidione. *Biochem. Biophys. Res. Commun.* 21: 523–530 (1965).

(*18*) Hoober, J. K., P. Siekevitz and G. E. Palade: Formation of chloroplast membranes in *Chlamydomonas reinhardi* y-1. Effects of inhibitors of protein synthesis. *J. Biol. Chem.* 244: 2621–2631 (1969).

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