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Short Note

New Observations on Green Hydra Symbiosis

Goran KOVAČEVIĆ, Mirjana KALAFATIĆ and Nikola LJUBEŠIĆ

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New observations on green hydra symbiosis are described. Herbicide norflurazon was chosen as a «trigger» for analysis of these observations. Green hydra (*Hydra viridissima* Pallas, 1766) is a typical example of endosymbiosis. In its gastrodermal myoeptihelial cells it contains individuals of *Chlorella vulgaris* Beij. (KESSLER & HUSS 1992). Ultrastructural changes were observed by means of TEM. The newly described morphological features of green hydra symbiosis included a widening of the perialgal space, missing symbiosomes and joining of the existing perialgal spaces. Also, on the basis of the newly described mechanisms, the recovery of green hydra after a period of intoxication was explained. The final result of the disturbed symbiosis between hydra and algae was the reassembly of the endosymbiosis in surviving individuals.

Key words: Green hydra, *Chlorella*, perialgal space, symbiosome, symbiosis reassembly.

Goran KOVAČEVIĆ, Mirjana KALAFATIĆ, Faculty of Science, University of Zagreb, Department of Zoology, Rooseveltov trg 6, HR-10000 Zagreb, Croatia,
E-mail: goran@zg.biol.pmf.hr
Nikola LJUBEŠIĆ, Ruđer Bošković Institute, Department of Molecular Genetics, Bijenička cesta 54, HR-10000 Zagreb, Croatia.

Symbiosis is one of the most important and most interesting subjects in evolutionary biology. In recent years this area of research was much revived, with new approaches and aspects revealed (HABETHA *et al.* 2003; OSBORNE & BERGMAN 2002; SECHBACH 2001). Different levels and areas of research were covered (KRAJČOVIČ *et al.* 2001; LÖFFELHARDT & BOHNERT 2001), but not the example of green hydra symbiosis. Symbiosis requires the harmonic coactions of a large number of genes in both symbionts. It is also connected with the eukaryotic cell symbiosis problem (EBRINGER & KRAJČOVIČ 1994; KRAJČOVIČ 2002). Research in this field contributes to the understanding of symbiosis from the complex and interesting relationships of the symbionts to different adaptations and coactions in the symbiosis assembly (BURNETT 1973; DOUGLAS 1994; HABETHA *et al.* 2003).

Green hydra (*Hydra viridissima* Pallas, 1766) is a well-known symbiotic and experimental object and has been studied for hundreds of years, with many symbiotic mechanisms already described (DUNN 1987; KALAFATIĆ 1995; KALAFATIĆ *et al.* 2001; KOVAČEVIĆ *et al.* 2001, 2003; MCAULEY *et al.* 1996; MEINHARDT 2002; PARDY & MUSCATINE 1973; RAHAT 1991; SHIMIZU & FUJISAWA 2003). Green hydra is a typical example of endosymbiosis. In its gastrodermal myoepithelial cells it contains individuals of unicellular green alga *Chlorella vulgaris* Beij. (KESSLER & HUSS 1992), each alga is contained in a vacuole called a symbiosome (BURNETT 1973; DOUGLAS 1994).

In this paper, new observations and morphological features of green hydra symbiosis are described.

Material and Methods

Green hydras were collected at the Jarun and Maksimir lakes in Zagreb, Croatia, from the surface of submerged plants. The organisms were maintained in the lab in glass dishes (11×5.5 cm) in aerated aquarium water, photoperiod 10 h light, 14 h dark, at room temperature (21°C). Animals were fed once a week with *Artemia salina* nauplia. Organisms were treated with five concentrations of aqueous solution of norflurazon (SAN 9789, Sandoz Ltd., Basel, Switzerland), $2x10^{-4}$, $2x10^{-5}$, $2x10^{-6}$, $2x10^{-7}$ and $2x10^{-8}$ mol/l.

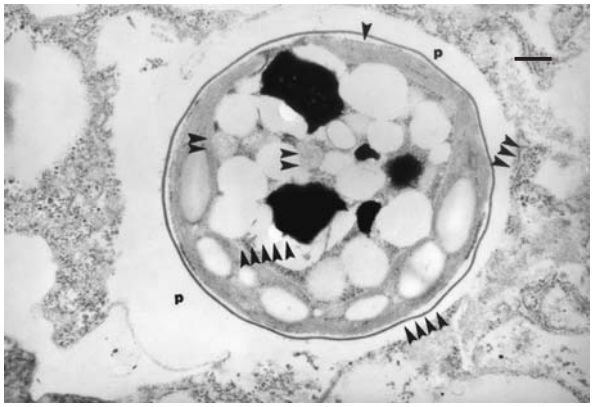

Fig 1. TEM of endosymbiotic alga *Chlorella vulgaris* Beij. (KESSLER & HUSS 1992) from green hydra (*Hydra viridissima* Pallas, 1766) treated with $2x10^{-5}$ mol/l of aqueous solution of nf. p – wide perialgal space. Damaged chloroplast (arrowhead) and mitochondria (2 arrowheads). Wrinkled cell wall (4 arrowheads). Osmiophilic globules (5 arrowheads). Fragmented rER (3 arrowheads). Bar = 0.5 μm.

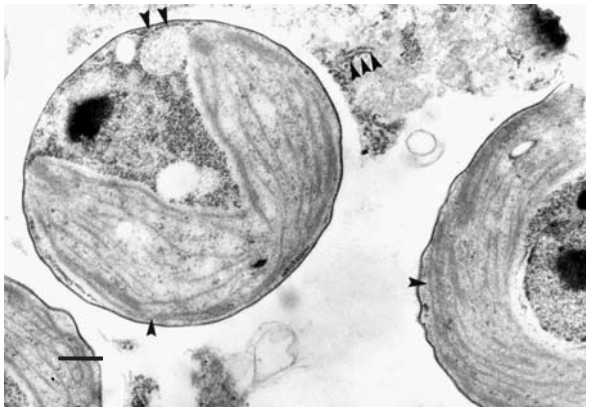

Fig 2. TEM of endosymbiotic alga *Chlorella vulgaris* Beij. (KESSLER & HUSS 1992) from green hydra (*Hydra viridissima* Pallas, 1766) treated with $2x10^{-5}$ mol/l of aqueous solution of nf. Algae entering into the perialgal spaces of other algae; joining of their perialgal spaces. Swollen chloroplasts (arrowhead) and mitochondria (2 arrowheads). rER torn apart (3 arrowheads). Bar = 0.5 μm.

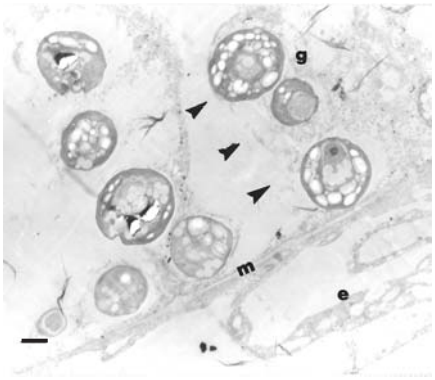

Fig 3. TEM of endosymbiotic alga *Chlorella vulgaris* Beij. (KESSLER & HUSS 1992) from green hydra (*Hydra viridissima* Pallas, 1766) treated with $2x10^{-8}$ mol/l of aqueous solution of nf, after the period of recovery. Regular assembly and formation of the algal «columns» (3 arrowheads) inside the gastrodermal myoepithelial cells (g) of green hydra – re-established symbiosis. m – consistent mesoglea. e – regenerating ectoderm. Bar =1 μm.

Standard preparation methods were used for TEM. Individuals from all the treated groups as well as the control were fixed on the third day after the beginning of the experiment. They were fixed in 2% glutaraldehyde, pH 7.2 buffered with 0.01% sodium-cacodylate buffer and postfixed in 1% osmium-tetroxide buffered with the same buffer. Afterwards, preparations were dehydrated, immersed into Spurr raisin and cut with a glass knife on an ultramicrotome. Sections were stained with 4% uranyl-acetate and lead-citrate (REYNOLDS 1963). Micrographs were made with electron microscopes Zeiss, EM10A and FEI Morgagni 269D, films Kodak and Imago.

Results and Discussion

Ultrastructural analysis of treated green hydras by TEM (KOVAČEVIĆ *et al.* 2001; KALAFATIĆ *et al.* 2001) showed new and interesting coevolutionary adaptations in green hydra symbiosis. Herbicide norflurazon (nf) was used as a "trigger" for the observations. By increasing the concentration of nf, changes and damage to green hydra were more intense, and the mechanisms of symbiosis became more obvious. For the first time several new findings on the symbiotic mechanisms in green hydra were described. First, wide, large and disturbed perialgal spaces were noticed around the endosymbiotic *Chlorella* (Fig. 1), compared to the control individuals that showed narrow and well-defined perialgal spaces. It seemed that this widening of perialgal spaces in the treated organisms presented a defensive and protective mechanism in the symbiosis. Algae "pushed away" the toxic effect of the xenobiotic. Further, symbiosomes of some algae were entirely missing, suggesting that symbiosomes had been removed or in some way degraded. In some individuals, algae even entered into the perialgal spaces of other green algae (Fig. 2), finally resulting in the joining of their perialgal spaces. This may have led to a more successful maintenance of the symbiosis and kept it active. It seems that this joining of the perialgal spaces could lead to the reassembly of the endosymbiosis in capable and surviving symbiotic *Chlorella* individuals. This could be observed in the green hydra individuals that managed to re-establish the symbiosis after a period of recovery and produce normal symbiotic interactions with algae again. A regular assembly of the algal columns inside the gastrodermal myoepithelial cells of green hydra were observed, as in the control organisms (Fig. 3).

The results of these experiments contributed to the resolving and better understanding of the complex problem of symbiosis. More detailed research concerning these, as well as other mechanisms of

green hydra symbiosis and "triggers" for their detection and observation need to be performed, using image analyzer programs, morphometry and other appropriate methods.

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