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Short communication

UVB radiation induces changes in the ultra-structure of *Iridaea cordata*

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ABSTRACT

Iridaea cordata cultivated in the presence of UVB radiation (UVBR) was studied using transmission electron microscopy. Apical segments were cultivated in 0.97 Wm^{-2} of UVBR for 40 days, 3 h a day, and compared to a negative control (UVBR absent). UVBR caused modifications, mainly in the cortical cells, including an increased number of cell wall-producing vesicles, in addition to thicker and denser cellular walls, compared to the control. Additionally, cells were observed with an irregular contour and without defined organelles. The increase of cell-wall thickness could be interpreted as an acclimation to UVBR, which could lead to protection from this radiation.

1. Introduction

Increasing levels of UVB radiation (280–315 nm) reaching the Earth's surface as a consequence of atmospheric ozone depletion have been reported annually since the late 1980s (Farman et al., 1985). This phenomenon has reached not only the Antarctic region, but also the southernmost part of South America, including Chile and Argentina (Kirchhoff et al., 1997; Diaz et al., 2006). Since ultraviolet radiation also penetrates the water column (Smith et al., 1992; Figueroa, 2002), marine organisms are exposed to its harmful effects, as well. Benthic macro-algae, in contrast to phytoplankton, are fixed and restricted to their growth sites, thus lacking the ability to avoid solar radiation, especially during low tide (Franklin and Forster, 1997).

UVBR have been found to affect marine macroalgae in several ways, including the effects on photosynthesis, nitrogen metabolism, growth, and DNA (reviewed by Bischof et al., 2006). While the effects of UVBR on the physiology of algae are well-documented, only a few papers have reported changes in the ultra-structure of macro-algae, and most of them evaluated only the short-term exposure to UVBR. Among them, Poppe et al. (2002, 2003) reported the formation of "inside-out" vesicles from the thylakoids in four red algae and, additionally, enlarged cristae in the mitochondria. Similar results were obtained for red algae, *Palmaria palmata* and *Odonthalia dentata,* which had wrinkled thylakoid membranes, dilatations of the thylakoid lumen, and an altered envelope, whereas the mitochondria had become damaged and plastoglobuli had formed (Holzinger et al., 2004). In contrast, the ultra-structure of the green alga, *Prasiola crispa*, was relatively stable after short-term exposure to UVBR (Holzinger et al., 2006). Results for the long-term exposure (28 days) showed changes in the ultra-structure of cortical and subcortical cells, which included increased thickness of the cell wall, changes in the cell contour, and destruction of the chloroplast internal organisation in *Kappaphycus alvarezii* (Schmidt et al., 2009).

Iridaea cordata is a cold-adapted red alga occurring from the Antarctic to sub-Antarctic coast (Cormaci et al., 1992; Wiencke and Clayton, 2002). This species inhabits hard substrates in the middle-upper intertidal and is exposed to extreme changes in environmental conditions daily. Therefore, this alga is exposed to increasing levels of UVBR and its harmful effects, especially during low tides. The species is considered an important carragenan-producing red alga (Craigie, 1990), and it has been harvested in the south of Chile, together with other carrageenan algae.

I. cordata has an erectus thallus, growing up to 30 cm tall, and consists of an expanded laminar region enlarging from a narrow stipe. In cross section, cortical and medullary regions can be easily distinguished (Foltran et al., 1996). The cortical region is formed by close rows of small, anticlinally arranged, roundish cells, whereas the medullary region has a loose network of irregular shaped cells embedded in a huge mass of translucent intercellular material (Navarro et al., 2003). Chloroplasts have a single peripheral encircling thylakoid and a variable number of evenly spaced thylakoids (Foltran et al., 1996; Navarro et al., 2003). Mitochondria with flattened cristae are found in close association with chloroplasts and Golgi bodies (Navarro et al., 2003). Golgi bodies are composed of a few stacked cisternae, and mitochondria are present in proxim-

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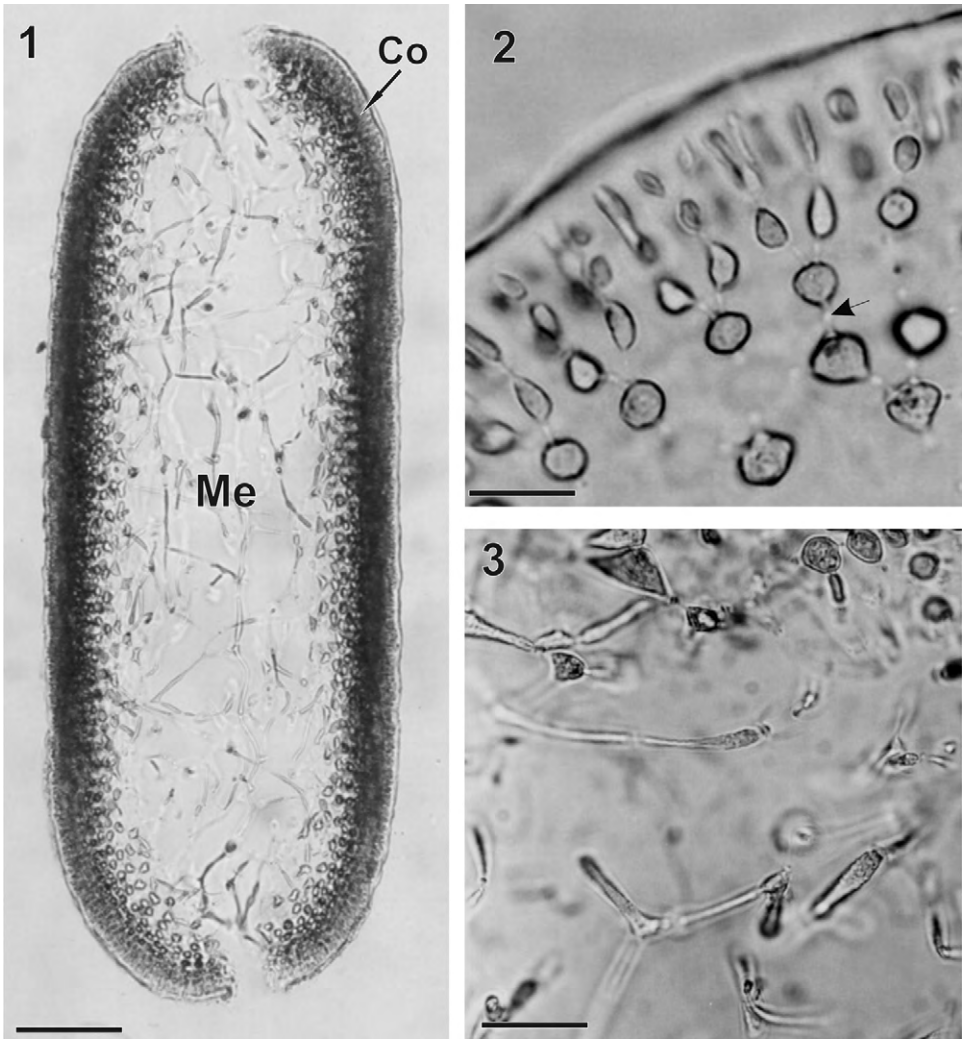


Figs. 1–3. Light microscopy: cross-sections of *Iridaea cordata* cultivated in control conditions; Fig.1. Cortical (Co) and medullary region (Me). Fig. 2. Cortical region showing close rows of small cells joined by pit-connections (arrow). Fig. 3. Medullary region composed of a loose network of irregularly shaped cells. Bar in (1) = 230 μm; bars in (2) and (3) = 30 μm.

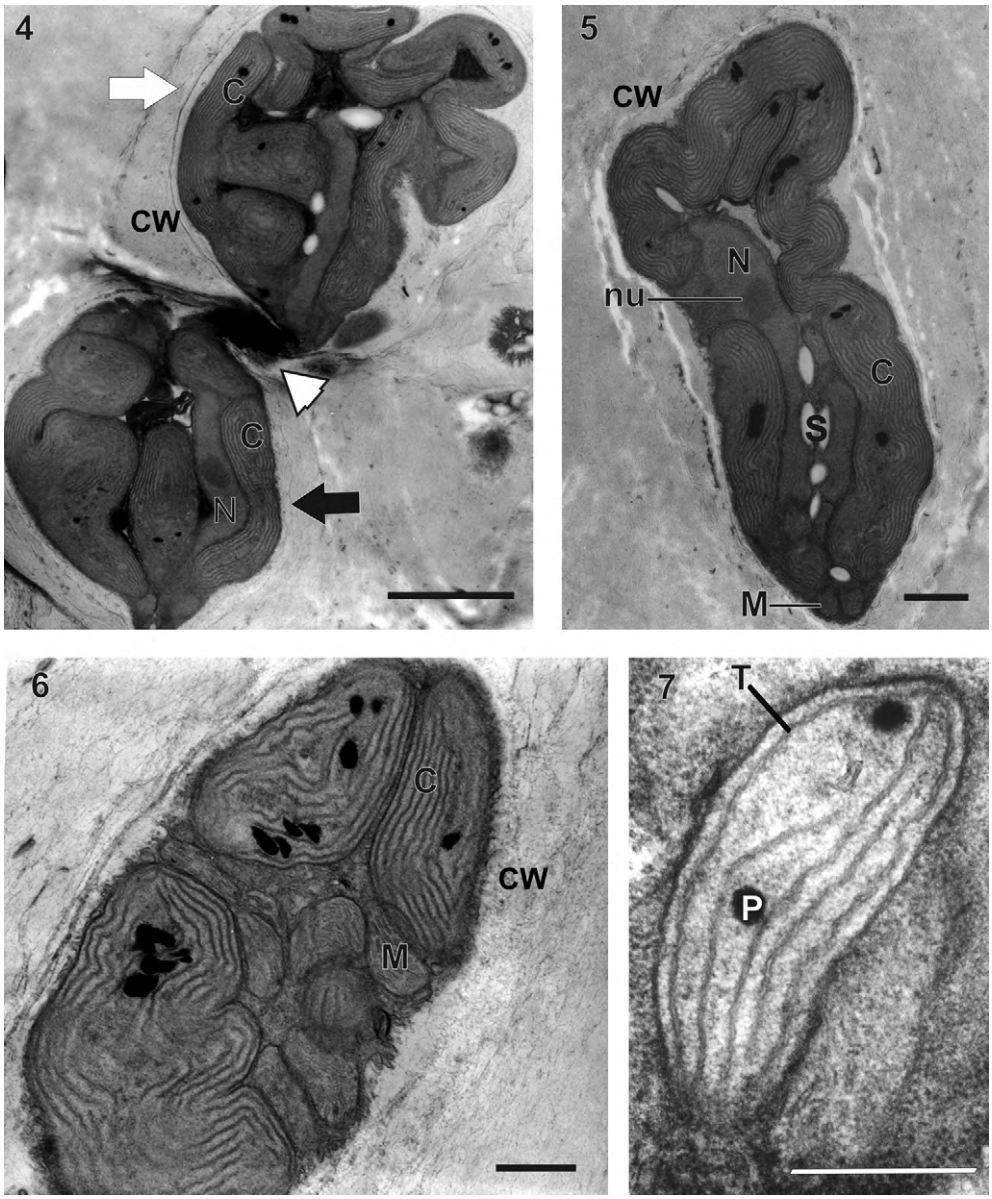
ity to the cis face (Navarro et al., 2003). Some compounds of the red alga cell wall are synthesised and wrapped in Golgi bodies and afterwards transported to the periphery of the cell by vesicles that release their contents out of the plasmalemma, after blending with the cell membrane (Ramus, 1972; Pueschel, 1990).

The effects of UVBR on *I. cordata* were evaluated according to the carpospore and germling mortality, growth rates of plantlets, and growth rates and pigment content of plants (Navarro et al., 2010). Moreover, spores were assessed for photosynthetic performance, mycosporine-like amino acid concentration, and DNA damage (Zacher et al., 2009). The aim of this study was to evaluate the effects of long-term exposure (40 days) to artificial UVBR (0.97 Wm^{-2}) on the ultra-structure of *I. cordata*.

2. Materials and methods

Fronds of *I. cordata* were collected from the intertidal zone of Posesión Bay (52°13′ S; 69°17′ W), Strait of Magellan (Chile), in January 2001, and transported to the laboratory. Because the apical sections of *I. cordata* are very irregular, 1-cm^2 fragments were selected from sub-apical section to standardise the biomass and area, and unialgal cultures were established as described by Oliveira et al. (1995). Cultures were maintained in Provasoli's enriched seawater (20 $mL L^{-1}$; 31 psu salinity), which was prepared without Tris phosphate (Ursi et al., 2008), in a temperature controlled room at 9 ± 1 °C and 55 μmol photons $m^{-2} s^{-1}$ PAR provided by Philips TLT 20W/54 daylight fluorescent tubes, on a 12 h light/12 h dark cycle. The medium was replaced weekly. Two treatments were performed (control and UVBR). PAR treatment (control) was similar to the general culture conditions, whereas in the other treatment, algae were exposed to the combination of three fluorescent tubes (PAR) and two TL 20W/12RS tubes (UVBR) (Philips, The Netherlands) for 3 h per day in the middle of the light period. Although the TL 20W/12RS tubes also emit UVAR radiation, the UVBR/UVAR ratio is larger (1.27) when compared to nature, which is 0.026 (UVBR = 0.73 Wm^{-2}, UVAR = 27.70 Wm^{-2}; daily average values of September–October 2009, Ozone Laboratory, Universidad de Magallanes), but was already reported as 0.14 on November 21, 1999 (Diaz et al., 2006).

UVBR and PAR were measured using a Photometer/Radiometer (Solar Light Company Inc., model PMA2200) connected to the UVBR and PAR detectors (Solar Light PMA2101 and Solar Light PMA2132, respectively): UVBR, 0.97 Wm^{-2} (10.48 $kJ m^{-2}$ daily dose); and PAR, 55 μmol photons $m^{-2} s^{-1}$ (516.24 $kJ m^{-2}$ daily dose). The UVAR radiation was available at 0.76 Wm^{-2} (7.96 $kJ m^{-2}$ daily dose). UVCR light was filtered with the cellulose diacetate foil (0.075-mm thick), which displayed 0% transmission below 286 nm.



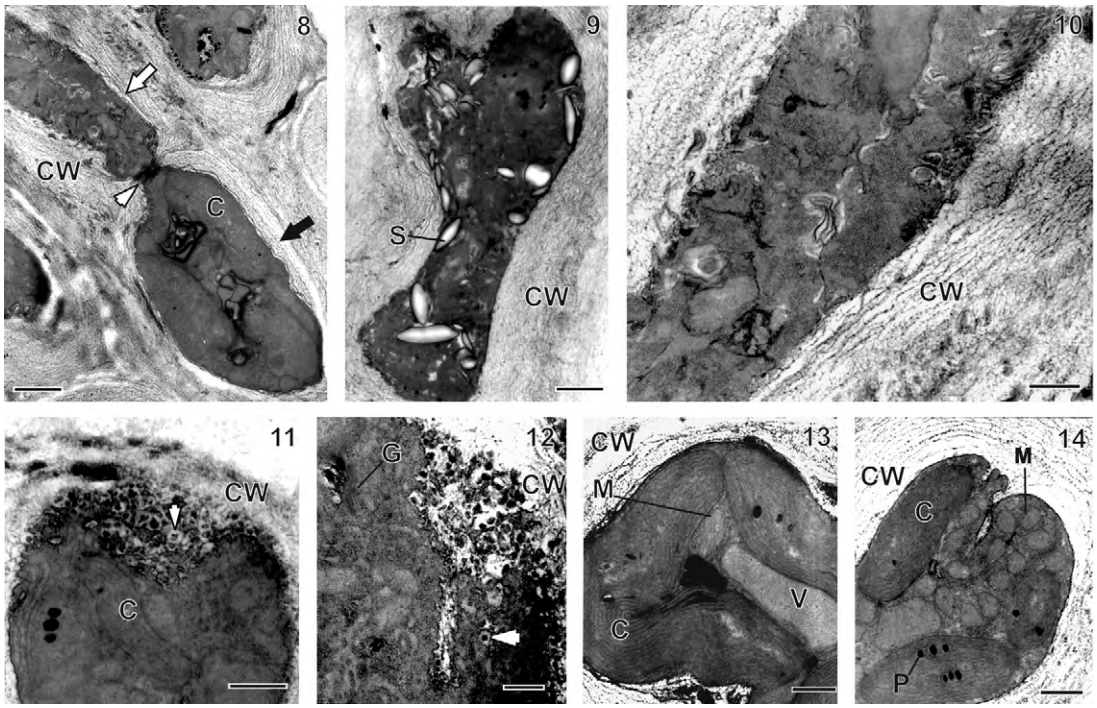
Figs. 4–7. Transmission electron microscopy: *Iridaea cordata* cultivated in control conditions. Fig. 4. Cells of second (white arrow) and third (black arrow) layers of the cortex joined by "pit-connections" (arrowhead). Fig. 5. Cortical cell. Fig. 6. Medullary cell. Fig. 7. Chloroplast of cortical cell with a single peripheral thylakoid. C, chloroplast; CW, cell wall; M, mitochondria; N, nucleus; nu, nucleolus; P, plastoglobulus; S, floridean starch grain; T, thylakoid. Bar in (4) = 4 μm; bar in (5) = 1.6 μm; bars in (6) and (7) = 0.8 μm.

Plants were cultivated in these conditions for 40 days, and two mm fragments were cut and prepared for transmission electron microscopy (Plastino and Costa, 2001; Navarro et al., 2003). Briefly, pieces of *I. cordata* were fixed in 3% glutaraldehyde, 0.2 M phosphate buffer, and 0.2 M sucrose at pH 7.0 for 19 h at 4 °C. After washing in phosphate buffer with decreasing concentrations of sucrose at 10 °C, the samples were post-fixed in 1% OsO_4 for 4 h at 4 °C. Afterwards samples were washed in 0.1 M phosphate buffer and 0.1 M NaCl at 10 °C, and maintained in 0.1 M NaCl for 18 h at 4 °C. The dehydratation was carried out in a graded acetona series before infiltration with the Spurr's medium. Thin sections were stained with 2% uranyl acetate and lead citrate. The material was examined using a Zeiss EM 900 microscope (Germany).

Cortical cells were analysed using the stereoscopic microscope and the Image Pro Plus 4.1 software. Their mean values for length and width, as well as their standard deviations were calculated from 30 measurements per treatment. The statistical significance of means was tested with a one-way ANOVA.

3. Results and discussion

The cross sections of *I. cordata* fragments cultivated in control conditions showed typical cortical and medullary regions (Fig. 1). The cortical region showed two or three layers of cells. In the outermost layer, the cells were elongated, whereas the second and third cell layers were spherical (Fig. 2). Cytoplasmic connections ("pit connections") between the adjacent cells were commonly observed (Fig. 2). The medullary region was composed of irregularly shaped cells (Fig. 3). At the ultra-structural level, cells of the outermost and the second layers of the cortex were surrounded by a thick cell wall (Figs. 4 and 5). Organelles like chloroplasts, nucleus, and mitochondria were commonly observed in both cortical and medullary cells (Figs. 5 and 6). The chloroplasts assumed the typical internal organisation of the red algae (Pueschel, 1990) consisting of a single peripheral thylakoid, while other thylakoids were unstacked and evenly spaced (Fig. 7).



Figs. 8–14. Transmission electron microscopy: *Iridaea cordata* exposed to UVBR (0.97 Wm^{-2}). Figs. 8–13. Cortical region. Fig. 8. Cells of the outermost (white arrow) and second layer (black arrow) of cortex joined by pit-connections (arrowhead). Outermost cell with irregular shape. Fig. 9. Outermost cell: floridean starch grains scattered in the cytoplasm. Fig. 10. Irregular cell shape. Fig. 11. Cell wall-producing vesicles (arrow). Fig. 12. Vesicle formation (arrow) and release of vesicle contents out of the plasmalemma. Fig. 13. Cell of the second layer of cortex with preserved organelles. Fig. 14. Medullary cell with chloroplasts, vacuoles, and numerous mitochondria. C, chloroplast; CW, cell wall; G, Golgi complex; M, mitochondria; P, plastoglobulus; S, floridean starch grain; V, vacuole. Bar in (8) = 4 μm; bar in (9) = 1.6 μm; bars in (10), (11), (12) and (13) = 0.8 μm; bar in (14) = 0.5 μm.

Mitochondria were present in association with the chloroplast (Fig. 6). This association was very apparent in the red alga cells (Pueschel, 1990) and probably plays an important role in the enhancement of metabolism (Oates and Cole, 1989).

Fragments of *I. cordata* exposed to UVBR became pale, and a necrosis was observed. Cells of the outermost layer of the cortex showed an increase in the values of length (control = 8.97 ± 1.59 μm, UVBR = 10.16 ± 1.49 μm; $F = 8.82$, $df = 58$, $P = 0.004$), but not the width (control = 5.8 ± 1.09 μm, UVBR = 6.2 ± 1.19 μm; $F = 2.47$, $df = 58$, $P = 0.121$). At the ultrastructural level, cells of these fragments showed pronounced changes (Figs. 8–14). The UVBR caused modifications, mainly in cells of the outermost layer of cortex directly exposed to the radiation, which showed an alteration in shape and an increase in the thickness and density of the cell wall (Figs. 8–10). Furthermore, these cells presented a great number of vesicles together at the external face of the thallus (Figs. 11 and 12), which could be responsible for the increase in cell wall (control = 2.13 ± 0.71 μm, UVBR = 2.86 ± 0.91 μm; $F = 8.48$, $df = 40$, $P = 0.006$). In this context, Günter and Ovodov (2007) showed that UVBR increased the biomass and the concentration of polysaccharides in the vascular plant callus. Nevertheless, UVBR caused a decrease of carragenan yield in *K. alvarezii* (Eswaran et al., 2001), but this study was conducted in short-term exposure to UVBR and might present misleading results because of an overestimation of UVBR effects, since acclimation processes was neglected. Our study, however, was conducted over a period of 40 days. This could allow *I. cordata* to synthesise more wall compounds. In *K. alvarezii* exposed to UVBR during a period of 28 days there was greater deposition of neutral polysaccharides, referred to as cellulose (Schmidt et al., 2009).

The alterations in shape of the *I. cordata* cortical cells could promote higher thickness of the thallus, as reported for leaves of higher plants (for a review, see Jansen et al., 1998). Possibly, the increase of superficial area of the cell walls occurred to scatter the high UVBR levels and prevent the radiation from reaching the more internal layers of the cortex. These alterations could be considered as part of a process of acclimation to the long-term UVBR exposure.

Although floridean starch grains scattered in the cytoplasm were observed, defined organelles could not clearly be recognised in some of the cortical cells of *I. cordata* directly exposed to UVBR (Fig. 9). Membrane alterations were already reported for chloroplasts and mitochondria of macroalgal cells exposed to short-term UVBR (Murphy, 1983; Poppe et al., 2003; Holzinger et al., 2004). The endo-membrane structures are thought to be sensitive to UVBR because of the reactive oxygen species (ROS), which breaks the lipids or molecules that make them up (Murphy, 1983; Kramer et al., 1991; Bowler et al., 1992).

Medullary cells and those of second and third layers of the *I. cordata* cortex were less damaged by UVBR than the outermost layer of the cortex. The former showed preserved organelles such as chloroplasts, vacuoles, and numerous mitochondria (Figs. 13–14). The lack of structural alterations in these organelles could also result from an efficient defense and protection mechanism, such as the mycosporine-like amino acids that had already been characterised in this species (Hoyer et al., 2001). Additionally, carotenoids, detoxicant enzymes, and antioxidants could contribute to this mechanism, detaining the destructive action of ROS, as has been demonstrated for the other green, red, and brown species (Aguilera et al., 2002).

Although, our study was not designed to perfectly mimic natural conditions and should be considered mechanistic, it could predict the effects of a long-term exposure to UVBR on the marine macro-alga ultra-structure. The higher UVBR/UVAR ratio of the TL 20W/12RS tube compared to the sun spectrum could simulate the effect of the ozone hole conditions. The high UVBR/UVAR ratio could have important photo-biological consequences because the UVBR has a disproportionally large biological effect and damages

macromolecules such as DNA, proteins, and lipids (Jones and Kok, 1966, Caldwell, 1971, Setlow, 1974, Franklin and Forster, 1997). This approach could be useful to predict the UVBR effects considering a worst ozone depletion scenario. An increase of the ultraviolet index by about 3–6% is expected in southern high latitudes in 2090–2100 (Hegglin and Shepherd, 2009), and this radiation will be additionally influenced by climate change (McKenzie et al., 2007).

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