Summary: Structural stress responses and degradation of dictyosomes in algae analysed by TEM and FIB-SEM tomography

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***Abstract*—The goal of this work is to summarize the paper of Lütz-Meindl et al. on their report of morphological alterations in the golgi apparatus of stressed algae species [1]. As model organisms served *Micrasterias* and *Nitella*, in whom the authors reported non-classical autophagy degradation of dictyosomes into single membrane balls, using transmission electron microscopy (TEM) and focus ion beam electron microscopy (FIB-SEM). Various different stress stimuli like metal intoxication, light starvation for 9 weeks, chemical inhibition of glycolysis (+photosynthesis) and disturbance of ionic homeostasis via KCl/Cd were thereby inflicted and the respective impacts observed. Common non-authophagy related dictyosomal degradation patterns were observed, with an increase in magnitude positively correlated to the severity of the threat. The described "ball" formation of the cisterna and the subsequent association with the ER membranes indicates a novel ER-dependent degradation pathway, at least in algae.**

***Key Words*—*Micrasterias*, *Nitella*, TEM, Golgi apparatus, FIB-SEM**

I. INTRODUCTION

Stress responses have been studied extensively, whereby chloroplasts, mitochondria, endoplasmatic reticula (ER) and dictyosomes have been identified as critical targets. Unfortunately, the body of studies was limited by the two-dimensional resolution of transmission electron microscopy, leaving questions, especially considering ER and dictyosomes, unanswered. Contrary to their dynamic nature, dictyosomes are stable and sensitive to stress, as demonstrated by inhibition of ER-transport and dissociation of COPI proteins. Golgi-centric stress stimuli, like of inhibition of N-glycosylation or Ca2+-ATPase, exposure to nitric oxide, hydrogen peroxide, metal ions and salt imbalances, have been shown to have an impact on morphological attributes of dictyosomes. Realizing, that the limitation of further research is partly based on appropriate models, Lütz-Meindl et. al adopted *Micrasterias denticulata* as a model, due to its high translational value to higher plants, constant number of cisterna (n=11, size 2-3μm, high contact to ER, low vesicle to surface ratio), large cellular size and delicate cell shape which is sensitive to environmental stress. Recent insights on three-dimensional dictyosomal and ER depictions, motivated the authors to decipher the stress-induced morphological phenomena with a refined scope, using FIB-SEM. Additionally the authors used TEM to investigate the experimental groups beforehand, amongst whom the salt imbalance stimuli (KCl) was also investigated in another algae species, *Nitella* (cisterna, n = 6-9, size=0.5-1μm, low contact to ER, vesicle to surface ratio high [1]). The detailed group assignments can be extracted under Materials and Methods.

II. METHODS AND MATERIALS

Briefly, the authors cultivated *micrasterias* to defined interphase stages (48h after mitosis) in Desmidiacean medium, while *Nitella* was cultivated in rainwater. The different *micrasterias* groups, were exposed to: 3mM MnSO4 for 7 days (1), to 150μM $CdSO_4$ for 4 h (2), or to 180, 200 or 300mM KCl for 3h (3-5) and 24h (6-8), 5 μM $Pb(NO_3)_2$ (9), complete darkness for 9 weeks (10), Diuron (0.5 μM, 14 days, photosynthesis inhibitor) (11) or 2-deoxy-D-glucose (50 μM, 21 days, glycolysis inhibitor) (12) or nutrient solution (negative control) (13). Correspondingly, *Nitella sp.* exposed to 180 mM KCl for 3h (14) and normal conditions (untreated control) (15). All samples were fixed by high-pressure freezing (Lütz-Meindl SOP), cryo-substituted (2% OsO_4 and 0.05% uranyl acetate, 60h -80°C, 4h -30°C), embedded in epoxy resin and sectioned via ultra-microtomy. The slides were mounted on copper grids (formvar coated) and all samples (1-15) were investigated at 80 kV via zero-loss energy filtering. Samples 2-5,13-15 were additionally investigated in FIB-SEM, with the following procedure: After embedding (as above) the mounted blocks were trimmed with glass using the mesa technique to allow lateral milling. The Ga-ion beam slicing thickness was 5-10nm (0.5–1 nA milling current, aperture 60 μm, high-current mode 1.5 kV of the in-lens EsB detector with the EsB grid set to 1000 V). Alignment of stacks was executed via ImageJ and Amira. The latter contributed to segmentation and 3-D reconstruction, which were corrected manually.

III. RESULTS

The authors showed a common degradation pattern under different mild stress conditions (groups 1,9,10,12), with cisternal curling, loss of cis-trans polarity, reduction in number of cisternae, lack or inhibition of vesicle formation and an increased number of multi-vesicular bodies. Depending on the severity of threat, cisternal detachment, indicating degranulation, was already observed in group 9, with even stronger effects in group 11 and 12 (also dilated ER cisternae were observed on the trans site of dictyosomes in this group) (see. Fig. S1). Ionic imbalances, as induced by Cd exposure (2) (disturbs Ca2+ homeostasis by binding to Ca2+ sites) show

[1] Dictyosome characteristics of *Nitella* and *Micrasterias* were confirmed in the positive controls by the authors.

the most severe pattern, similar to group 12, whereby the additional FIB-SEM information revealed a ball structure of the curled outermost cisternae. A more extreme remodeling was observed in the KCl groups (3-5), with an absolute loss of cis-trans polarity, absence of vesicles and persistent contact with trans side ER. The compartments were enlarged depending on degree of disintegration (Fig. S2). In groups 6-8, dictyosomes are no longer visible in TEM, but only large vacuoles containing smaller ones. The *Nitella* group (14) showed similar morphological phenomena, but less reliable ball formation.

IV. DISCUSSION

The presented data, illustrates a common non-autophagy dictyosomal degradation mechanism. The ball formation and consequent contact with the ER and the associated volume increase, might hint to an intake of the degraded cisternal balls to the ER. Starting from the cis and trans side, ball formation occurs and persists, depending on the stress levels, until the dictyosome is degraded into 5-6 balls. Subsequently, the balls associate with the ER membrane, where they appear to be absorbed (see. Fig. S3). The authors stress and attest the importance of FIB-SEM for these observations, with conventional TEM, discriminability to autophagy associated degradation is drastically lower.

APPENDIX
SUPPLEMENT

All Figures were taken from the original publication and serve as representative examples for the totality of evidence.

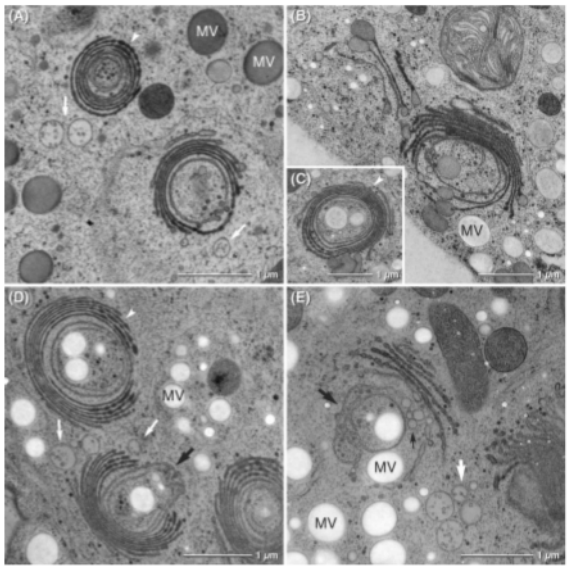

Figure S1: Abnormal structure and morphology of dictyosomes in Micrasterias induced by different abiotic stressors (moderate stress). (A) 3 mM MnSO4 for 7 days, (B,C) 5 μM $Pb(NO_3)_2$ for 21 days, (D) 9 weeks complete darkness and (E) 50 μM 2-deoxy-D-glucose for 21 days. White arrowheads point at dictyosomes without cis-trans-polarity, white arrows at multi-vesicular bodies, black arrow at partly (D) or completely detached (E) dictyosomal cisternae, small black arrow at ER. MV mucilagevesicles.

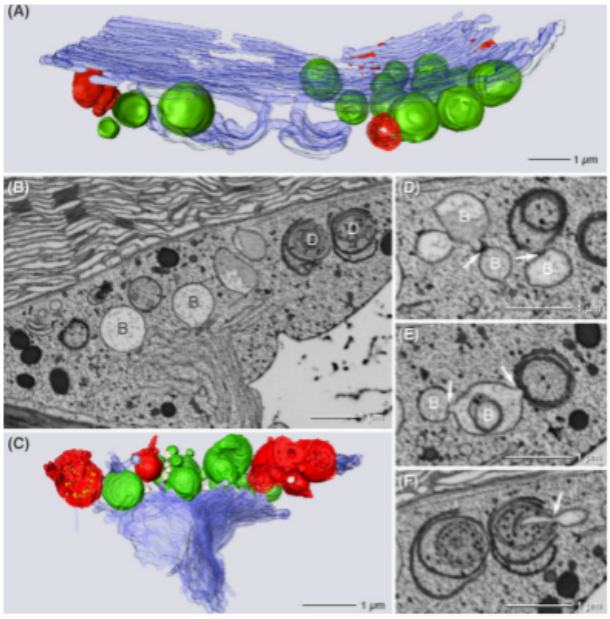

Figure S2: Membrane connections between different stages of dictyosomal degradation in Micrasterias after exposure to 180 mM KCl for 3 h, visualized by 3-D reconstructions (A,C) and by micrographs of FIB-SEM series (B,D,E,F). (A) Distribution of membrane balls (green), dictyosomal remnants (red) and ER (blue). (B) Contacts between ER and membrane balls are visible. (C) Later stage of dictyosomal degradation. Membrane balls (green) are connected to ER cisternae (blue). (D,E) Membrane contacts between balls (arrows) and dictyosomal remnants. (F) ER cisternae (arrow) penetrating dictyosomal remnant.B,membrane balls;D,dictyosomal remnants.

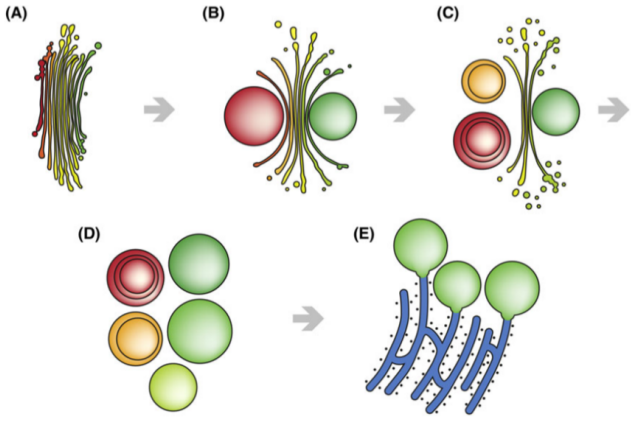

Figure S3: Schematic drawing of dictyosomal degradation after intense stress in Micrasterias. Cis-side in red, trans-side in green.(A) Unaffected dictyosome with 11 cisternae. (B) Dicytosomal degradation has started, outermost cis-andtrans-cisternae form balls, the number of "normal" cisternae is reduced. (C) Proceeding dictyosomal degradation. Cisternal balls incorporate other cisternal ball in them. The number of "normal" cisternae is further reduced; numerous small vesicles representing degradation products surround the dictyosome.(D) Normal dictyosomal architecture is completely lost.The former dictyosome consists only of cisternal balls with increased volume.(E) The cisternal balls fuse with long ER strands that have formed during stress.

REFERENCES

[1] U. LÜTZ-MEINDL, M. Luckner, A. Andosch, and G. Wanner, "Structural stress responses and degradation of dictyosomes in algae analysed by tem and fib-sem tomography," *Journal of Microscopy*, vol. 263, no. 2, pp. 129–141, 2016.