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PCD and autophagy in the unicellular green alga *Micrasterias denticulata*

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Abstract

Programmed cell death (PCD) plays a central role in normal plant development and is also induced by various biotic and abiotic stress factors. In the unicellular freshwater green alga *Micrasterias denticulata* morphological and biochemical hallmarks such as the appearance of autophagosomes, increased production of ROS and degradation of genomic DNA into small fragments ("DNA laddering") indicate PCD. Our data not only demonstrate that Micrasterias is capable of performing PCD under salt stress, but also that it is triggered by the ionic and not osmotic component of salinity. Additionally, results from the present and previous studies suggest that different inducers may lead to different cell death pathways in one and the same organism.

Keywords

autophagy; green alga; *Micrasterias denticulata*; programmed cell death; ROS; salt stress; ultrastructure

Over the past few years, it became evident that unicellular organisms such as green algae, dinoflagellates, yeasts, kinetoplastids, bacteria and cyanobacteria are capable of performing PCD, indicating that it emerged early in the evolution of higher eukaryotes.

The program which ultimately leads to self-destruction of the cell seems to be quite diverse, ranging from autophagy, apoptosis and necrosis to intermediate forms of these processes, and has led to a vibrant debate in the literature.

Recent investigations have focused on the question as to whether the freshwater green alga Micrasterias is capable of undergoing PCD upon abiotic stress, and which physiological, morphological and biochemical processes are involved. Salt stress as an inducer of PCD was chosen, as Micrasterias is naturally growing in acidic peat bog ponds with frequent changes in the concentration of the surrounding medium either by evaporation due to sunlight exposure or to dilution by rain. Moreover, Micrasterias has been used as a cell biological model system for many years, and thus comprehensive knowledge on cell differentiation, growth and physiology of this theoretically immortal green alga is already available.

In Micrasterias, NaCl and KCl induce DNA laddering which is a typical hallmark of PCD both in animals and plants. In contrast to salt stress, iso-osmotic sorbitol stress does not

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produce ladder-like degradation of DNA, suggesting that the ionic rather than the osmotic component of salt stress leads to the activation of endonucleases. This indicates a similar response of unicellular algae to ionic stress as reported for higher plants. The abrogation of DNA laddering in Micrasterias after pretreatment with the divalent cation Zn^{2+} points towards the involvement of Ca^{2+}-dependent endonucleases which are inhibited by Zn^{2+}. As Zn^{2+} is also a potent blocker of various ion channels, it may therefore indirectly lead to abolishment of DNA laddering.

To determine morphological changes that may accompany different types of PCD, light and transmission electron microscopy (TEM) investigations were employed in Micrasterias.

The most striking observation was the appearance of autophagy in TEM analyses after salt but not after osmotic stress. The autophagic process started by swelling of ER compartments, which subsequently began to surround dictyosomes and other organelles eventually leading to autophagosomes. Morphology of these autophagosomes strongly resembles that typically found in yeast, higher plants and animal cells. To our knowledge, our results provide the first evidence for autophagy during PCD in a unicellular alga.

Besides being an important mechanism in self-destruction of the cell during PCD, these autophagosomes could play a pivotal role in removing damaged organelles in order to maintain cellular homeostasis under ionic stress when the cells are still viable. Additionally, studies have shown that autophagy is also involved in degrading oxidized proteins during oxidative stress. A similar mechanism could exist in Micrasterias, as salt stress led to an increased production of reactive oxygen species (ROS). Although an increase in intracellular ROS could also be detected after osmotic stress, the different kinetics point to different pathways of stress response. Whereas salt stress induces an immediate boost in ROS production, which declines later on, osmotic stress increases ROS production with time. These differences could be reflected in the appearance of autophagosomes during salt but not osmotic stress.

Other ultrastructural changes during salt stress, such as the occurrence of balloon-shaped protrusion of the outer mitochondrial membrane, seem to be related to inner-osmotic changes within the organelles rather than to a functional disturbance. Respiration measurements demonstrated that mitochondria, despite their severe morphological abnormalities, are still physiologically active. This seems to be in contrast to the role of mitochondria in apoptosis of animals, where mitochondrial membranes rupture and apogenetic factors are released.

Caspases, a group of cysteine-proteases participating in PCD pathways of animals have not been found in plants so far. However, caspase-like activities have been reported in various vascular plants and green algae like Chlorella and Dunaliella. In Micrasterias, an increase in caspase-3-like activity could be detected during PCD after H_2O_2 treatment yet not after salt or osmotic stress. This indicates that different biochemical pathways are activated and lead to different forms of PCD in one and the same organism depending on the stress applied.

The appearance of the biochemical and morphological changes described above were accompanied by an active metabolism as indicated by cell viability, pigment composition, photosynthesis and respiration. This confirms that cell death in Micrasterias after salt stress is programmed, but not accidental or necrotic.

Taken together, our results show that not only is the theoretically immortal, unicellular green alga Micrasterias capable of performing autophagic PCD under salt stress, but the ionic rather than the osmotic component of salinity triggers this self-destruction of the cell. PCD as a response to salt stress has been also described in root cells of higher plants as an

adaptation process to adverse conditions. The reason why a unicellular photosynthetic organism like Micrasterias commits suicide under unfavorable environmental situations may be different from that of cells in tissues of higher plants. In the case of unicellular green algae, an altruistic PCD could be favorable for the whole colony in terms of selection of the fittest.

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