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Review

Hemoglobins, programmed cell death and somatic embryogenesis

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ABSTRACT

Programmed cell death (PCD) is a universal process in all multicellular organisms. It is a critical component in a diverse number of processes ranging from growth and differentiation to response to stress. Somatic embryogenesis is one such process where PCD is significantly involved. Nitric oxide is increasingly being recognized as playing a significant role in regulating PCD in both mammalian and plant systems. Plant hemoglobins scavenge NO, and evidence is accumulating that events that modify NO levels in plants also affect hemoglobin expression. Here, we review the process of PCD, describing the involvement of NO and plant hemoglobins in the process. NO is an effector of cell death in both plants and vertebrates, triggering the cascade of events leading to targeted cell death that is a part of an organism's response to stress or to tissue differentiation and development. Expression of specific hemoglobins can alter this response in plants by scavenging the NO, thus, interrupting the death process. Somatic embryogenesis is used as a model system to demonstrate how cell-specific expression of different classes of hemoglobins can alter the embryogenic process, affecting hormone synthesis, cell metabolite levels and genes associated with PCD and embryogenic competence. We propose that plant hemoglobins influence somatic embryogenesis and PCD through cell-specific expression of a distinct plant hemoglobin. It is based on the premise that both embryogenic competence and PCD are strongly influenced by cellular NO levels. Increases in cellular NO levels result in elevated Zn^{2+} and reactive-oxygen species associated with PCD, but they also result in decreased expression of MYC2, a transcription factor that is a negative effector of indoleacetic acid synthesis, a hormone that positively influences embryogenic competence. Cell-specific hemoglobin expression reduces NO levels as a result of NO scavenging, resulting in cell survival.

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1. Introduction

Two compounds, nitric oxide and hemoglobin, have an established history in biological research. Their role as significant factors in plant growth and development began in the last decade of the twentieth century. Prior to 1996 [1], there was little published information of studies on nitric oxide and its function in plants. Leghemoglobins had been known for over half a century, but it was only in the late 1980s that the existence of another type of plant hemoglobin was suspected as the result of the discovery of a hemoglobin in *Trema tomentosa*, a non-nodulating plant [2]. After this initial discovery, it took another six years before a hemoglobin gene, having only 40 percent nucleotide sequence identity with leghemoglobin gene, was isolated from barley [3], confirming the existence of a plant hemoglobin, distinct from leghemoglobin, with

Abbreviations: Hb, hemoglobin; PCD, programmed cell death; PEM, proembryogenic masses; ABA, abscisic acid; IAA, indoleacetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid.

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no function in a symbiotic relationship. Today, such hemoglobins have been identified in over 50 plant species [documented in ref. [4]].

The relationships among hemoglobins, NO and programmed cell death (PCD) are familiar topics in mammalian research, but it is only in the last two decades that research linking NO with PCD in plants has appeared. In this review, we will provide a summary of the current status of our understanding of plant PCD, the relationship of PCD with NO and the modulation of NO levels by hemoglobins. We will outline a hypothesis proposing that PCD is regulated by hemoglobin through modulation of cell NO levels.

2. Regulation of programmed cell death in plants

Emerging evidence suggests programmed cell death (PCD) is an integral component of plant development necessary to shape the body of the organism in conjunction with cell proliferation and differentiation. Implementation of PCD, which is triggered in response to several stimuli, including oxygen deprivation and other environmental stresses, occurs through precise cytological and molecular events sharing remarkable similarities with those observed in animal systems [5]. As reviewed [6], the process of PCD in eukaryotic cells can be divided into apoptotic death and autophagic death. While apoptotic death results from phagocytic cells degrading isolated or small groups of target cells, autophagic death eliminates larger clusters of cells or entire tissue and/or organs. The presence of rigid cell walls and lack of phagocytic cells make autophagic death the primary mechanism of PCD in plants.

2.1. Structural and ultrastructural features of autophagic cell death

Autophagic death in plants involves the sequestration of cytoplasm into autophagic vacuoles followed by their fusion with lytic vacuoles [7]. The formation of lytic vacuoles is poorly understood in plants. These vacuoles contain various enzymes comprising several hydrolytic enzymes, such as aspartate and cysteine proteases as well as nucleases [8]. In addition to newly formed lytic vacuoles, the storage vacuoles in cells dedicated to nutrient reservoirs, such as endosperm and cotyledon cells, are often converted into lytic vacuoles, leading to the hydrolysis of storage proteins [9]. Autophagic cell death can occur through non-disruptive and disruptive mechanisms. The non-disruptive mechanism involves the fusion of the tonoplasm (vacuolar membrane) with the plasma membrane resulting in the discharge of vacuolar enzymes in the apoplast. The disruptive process is triggered by the collapse of the tonoplasm and release of vacuolar enzymes within the cytoplasm [10]. The temporal development and subsequent collapse of the lytic vacuoles is best exemplified during aerenchyma formation, where it occurs in three distinct phases [11]. The biogenesis of lytic vacuoles can be de-novo or through modifications of pre-existing storage vacuoles. The lytic vacuoles of cells targeted by PCD increase in size and occupy the majority of the cell volume in phase 1, until only a reduced layer of the cytoplasm is retained. These changes precede the invagination of the tonoplast and the subsequent sequestration and degradation of the cytoplasm into the lumen in phase 2 [8]. These events are reminiscent of micro and macro-autophagy observed in animal cells, accompanying the early phases of PCD [12]. The final step involves the rupture of the tonoplast and the release of vacuolar enzymes, which further degrade cellular components. The enzymatic degradation starts with the endoplasmic reticulum, followed by the nucleus and mitochondria [8]. Variations in the sequence of these events are often documented, such as in the case of rice aerenchyma formation, where the disruption and degradation of the cell wall precedes the collapse of the lytic vacuole [13].

Programmed cell death-induced cell dismantling is characterized by stereotypic changes in nucleus and cytoskeletal organization. As reviewed [6], events occurring in the nucleus are prominent features in PCD. Nuclear disintegration followed by degradation of DNA, chromatin condensation and nuclear fragmentation are well characterized events, not only during apoptosis in animals but also autophagic cell death in plants. These events contribute to the death pathway by preventing DNA replication and transcription [14]. Systemic fragmentation of DNA is generally a two-step process involving two different types of nucleases [15]. During the initial phases of PCD the DNA is cleaved at the inter-loop sites of the chromatin yielding fragments of about 50–300 kbp. This is soon followed by the appearance of smaller DNA fragments resulting from the activity of the endonuclease enzyme, DNase1 [16]. Different fragmentations patterns are often observed among species [17,18], possibly due to the non-selective nature of vacuolar nucleases in the digestion of the nucleosomes (reviewed in [10]). DNA fragmentation and condensation of the chromatin are two processes occurring concomitantly, which can be redistributed along the periphery of the nucleus [19]. Nuclear fragmentation has been described as one of the last steps of PCD, following the collapse of the vacuolar membrane and protein hydrolysis within the cytoplasm. Variations in this sequence are observed, however, such as the case of aerenchyma formation in oxygen-deprived *Sagittaria lancifolia* plants where nuclear fragmentation appears to be an early event of PCD [20].

Comprehensive variations in cytoskeletal structure during PCD in plants have also been described [21]. Specifically, dissociation of microtubule-associated proteins are required during the early phases of autophagic cell death and contribute to changes in the actin network. The microtubular proteins may provide a scaffold for the movement of components triggering autophagy, as described in animal systems [22].

2.2. Cell death during embryogenesis

The early manifestation of cell death is observable in both animal and plant embryogenesis, where the process ensures the removal of "undesired" cells during shaping of the embryos. Compared to their animal counterparts, plant embryos have a simple organization and are composed of a few tissue types: pro-vascular tissue comprised of ground tissue and protoderm along the radial axis; shoot and root apical meristems; the hypocotyl along the apical-basal axis [23]. Due to this simple arrangement, PCD plays a less pronounced, but yet determinant role during plant embryogenesis and it is not surprising that plant embryos have become a suitable model system to study cell death. PCD fulfills three crucial roles during in vivo plant embryogenesis [6]. Firstly it eliminates the suspensor, the organ pushing the developing embryos into the endosperm cavity and responsible for transporting nutrients and growth factors from the maternal tissue into the embryo. The suspensor is not needed during late embryogenesis and it is therefore removed by PCD [24]. Cell death occurs during monozygotic polyembryony, characterized by the formation of multiple embryos per seed. Execution of the death program in all but one embryo results in the survival of the dominant embryo [25]. PCD also takes place during more advanced phases of embryogenesis and it is manifested as xylogenesis in the formation of death tracheary elements in the metaxylem [26], which are integral components of a functional vascular tissue. Hbs have been detected in tracheary elements of differentiating tissue of rice during seed germination [27]

Studies of PCD during early embryogenesis are difficult to perform due to the nature of the seed embryo, which is embedded in maternal tissue and, therefore, difficult to dissect. The somatic