HAVE NECROHORMONES A RÔLE IN EMBRYOGENESIS?

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ABSTRACT

The recognition of apoptosis (programmed cell death) as an accompaniment of normal development, the products released by the protoplasts undergoing self-destruction being utilized by adjacent living cells, stimulates renewed interest in Haberlandt's concept of "necrohormones" playing a role in apomictic reproduction. Recent work on somatic embryogenesis in carrot shows that regular death of certain cells in embryogenic cultures satisfies the criteria of apoptosis. Similar observations have been made with embryogenic cultures of *Picea abies*. Haberlandt's claim that cell death induced by injury adjacent to an ovule in *Oenothera* could lead to parthenogenesis, despite conflicting evidence from later experimenters, is worthy of reexamination.

KEY WORDS: necrohormones, embryogenesis.

In the early years of this Century Haberlandt provided impressive evidence that wounded cells released a substance or substances which stimulated cell division in adjacent intact cells. These substances were termed "wound hormones" (Haberlandt, 1923). Although Haberlandt's first experiments were with somatic storage tissues, such as slices of potato tuber and kohlrabi, he came to enquire whether similar stimuli from dead or dying cells could bring about development of unfertilized egg cells. From here it was a natural progression to consider whether influences from dying tissues could play a regular and determinative role in apomictic reproduction.

Haberlandt's first experiments with normal reproductive systems were made with *Oenothera lamarckiana* (Haberlandt, 1921a). The flowers chosen for the experiment were castrated and the ovaries then either crushed between thumb and forefinger, or punctured with a fine steel or glass needle. The object of this maltreatment was to cause release of wound hormones in the vicinity of embryo sacs. Haberlandt indeed found evidence of cell division and callus-like developments in the nucelli of several ovules in the damaged regions, and occasionally extended nuclear division within embryo sacs. In two cases there appeared to be parthenogenetic development of the egg cell, although the material had been fixed before the clear development of an embryo. More frequent were outgrowths of the nucellus into the cavity of the embryo sac. In several cases these took on the character of nucellar embryos.

Apart from these experimental results, Haberlandt also drew attention to the fact that dying cells were often a feature of sites of apomictic reproduction (Haberlandt, 1921b). In *Taraxacum*

officinale, for example, the embryo sac is surrounded by a single layer of cells (derived from the inner layer of the integument, and referred to as the tapetum) which becomes distinct by virtue of the abundant cytoplasm in its cells and their thin walls. Cells in this layer begin to die either just before, or concurrently with, the formation of the egg apparatus in the sac. Haberlandt suggested that these degenerating cells were the source of "necrohormones" which stimulated the development of the unreduced egg cell. Significantly, in the related and amphimictic *Hypochaeris radicata* there is no corresponding degeneration of the tapetal cells. Degeneration is also absent from the amphimictic genera *Lactuca* and *Sonchus*. A comprehensive statement of Haberlandt's views soon followed (1922).

The reaction to Haberlandt's experimental results and speculations was mixed. Hedemann (1931) made puncture experiments with *Oenothera gigas* and obtained nucellar embryos absent from the controls. No parthenogenetic egg cells were detected, and puncturing of the sac itself usually led to death of the whole ovule. Hedemann extended the experiments to other genera, but the results were not impressive. With *Mirabilis*, in which the ovary contains a single ovule, of 200 emasculated flowers, each ovary punctured 1-5 times with a fine needle, or alternatively squashed, only 50 remained healthy. Of these one yielded an undoubted embryo; others showed various irregular responses to the wounding. Subsequently, Beth (1938) repeated Haberlandt-type experiments on *Oenothera lamarckiana*, and extended the experiments to several other species of both mono- and dicotyledons, with wholly negative results. He concluded that such success as Haberlandt and Hedemann had obtained could be put down to inefficient castration, and that the evidence for wound or necrohormones

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being able to stimulate either parthenogenesis or adventive embryony was not convincing. No general theory of nucellar embryony following from the influence of dying cells in the ovule was, in Beth's view, tenable.

Although Haberlandt (1938) responded vigorously to many of Beth's more serious criticisms and defended his position in general (while allowing that necrohormones might provide only a contributory, rather than a principal, cause of apomixis), the concept of necrohormones having a rôle in reproduction receded from current thinking. Nevertheless, the recent discoveries of situations in which dying cells appear to participate regularly in somatic embryogenesis has renewed interest in the significance of cell death accompanying the re-establishment of embryogenic activity. In carrot somatic embryogenesis, for example, Pennell et al. (1992) have shown that in embryogenic suspension cultures the small spherical cells which have the highest embryogenic potential (first recognized by Nomura and Komamine (1985)) possess a wall with a uniformly modified pectic composition, detectable by their binding the monoclonal antibody JIM8. When these cells divide, they give rise to a non-reactive daughter cell, the two cells frequently remaining attached. The fates of the two cells are however very different. The JIM8-reactive parent cell dies, but the non-reactive daughter continues to divide and gives rise to the proembryogenic mass.

Participation of degeneration in somatic embryogenesis has also been observed in *Picea abies*. In single cell cultures obtained from cleaving embryonal suspensor masses a proportion of the cells behave as central cells in developing archegonia. The nucleus divides, but not the cell. One nucleus degenerates, while the viable nucleus accumulates an organized cytoplasm. Released into the medium, these cells (which are diploid) behave as fertilized egg cells. Free nuclear division is followed by the organization of an embryo (Durzan et al., 1994).

These two examples of somatic embryogenesis significantly involve death of associated cells (or disorganization of nuclei). The possibility at once arises that, as a consequence of this death and disorganization, embryogenic potential is released in the viable cell (Bell, 1994). It follows that this death and disorganization is an intrinsic part of the developmental programme. It is not accidental or sporadic. Programmed death (known as apoptosis) of certain cells is now recognized as an important element in animal development (Kerr et al., 1972; Raff, 1992). It follows from the activation of specific genes, which are now being identified (Steller, 1995), and involves depolymerization of nuclear DNA by endonucleases and of ubiquitin-tagged proteins by proteases (Havel and Durzan, 1995b).

The degenerative processes involved in the somatic embryogenesis occurring in both carrot and *Picea* have now been shown to have the cytochemical characteristics of apoptosis as it is known in animal systems (McCabe et al. 1996; Havel and Durzan, 1995a, b). It seems inevitable that the digestion products released by cells undergoing apoptosis will become available to the adjacent cells in which no programme of self-destruction has been activated. These products may well have morphogenetic effects, particularly if they include sugar fragments (oligosaccharides). These are known to have remarkable properties, including the ability to stimulate meristematic activity (Aldington and Fry, 1993), and in leguminous plants nodule formation (Lerouge et al., 1990).

A RÔLE FOR APOPTOSIS IN APOMIXIS?

Certainly the investigations of apomictic ovules since Haberlandt's time have reinforced his speculations. In *Alchemilla*, for example, in which aposporous apomixis predominates, Izmailow (1986) comments on the extensive degeneration of the archesporial cells (including those in which meiosis is attempted) not involved in the formation of the aposporous sacs. In *Ranunculus auricomus* (Nogler, 1984) the formation and development of the aposporous sac coincides with the degeneration of the meiotic megaspore, and in *Cortaderia jubata* (Gramineae) with the degeneration of the megaspore mother cell or its meiotic products (Philipson, 1978).

The increasing understanding of apoptosis as a precisely controlled process encourages a fresh look at these situations.

It seems now to be emerging that Haberlandt's thinking was indeed along the right lines, but his experimental techniques were altogether too crude for the problem he was attempting to resolve. The release of substances which promote embryogenic development may well occur in apomictic reproduction, but as a consequence of a genetically controlled sequence of events in certain cells, causing their apoptotic self-destruction. This would lead to a localized release of digestion products within intact tissue, the whole process being free from the traumatic and multiple effects of wounding. In the case of aposporous apomixis the self-destruction of the meiotic cells may provide the embryogenic stimulus, as already suggested by Mogie (1992), apparently unaware of Haberlandt's work. Cases are known (for example, in *Ranunculus auricomus* (Nogler, 1971)) where degeneration of the meiotic cells fails to be accompanied by the formation of aposporous sacs. This may indicate that it is necessary for the nucellar cells to acquire some kind of competence before they can respond to the influences from the degenerating meiotic cells. It is not clear whether aposporous sacs can arise in the absence of degenerating meiotic cells. If not, then the arguments for the degenerating cells having a Haberlandt-like effect are correspondingly strengthened.

It is not difficult to envisage how a genetic system leading to aposporous apogamy might have arisen. It has already been argued elsewhere (Bell, 1996) that the selective death of three of the spores in the normal megaspore tetrad of the angiosperms can be attributed to an apoptotic programme being activated in each of these three spores. In the scheme proposed (which depends upon the sporophyte being heterozygous at two loci, with obligate crossing over) the programme is not activated in the sporophytic condition. A modification of this system leading to the apoptotic programmes being activated, in the conditions of the ovule, in all four genotypes represented in the megaspore tetrad would lead to the situation seen in aposporous apomixis, the substances released by the self-destruction of the meiotic cells leading, as envisaged by Haberlandt and Mogie, to embryo sac formation and embryogeny in the adjacent nucellus.

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CZY NEKROHORMONY ODGRYWAJĄ ROLĘ W EMBRIOGENEZIE?

STRESZCZENIE

Apoptoza (zaprogramowana śmierć komórki) może być uważana za przejaw normalnego rozwoju organizmu ponieważ składniki uwolnione z protoplastów przechodzących proces samodestrukcji są wykorzystywane przez przyległe, żywe komórki. Taki pogląd ponownie czyni aktualnym koncepcję Haberlandta, że nekrohormony odgrywają ważną rolę podczas rozmnażania apomiktycznego. Najnowsze prace nad embriogenezą somatyczną marchwi wykazały, że śmierć pewnych komórek w kulturach embriogenicznych przebiega zgodnie z kryterium apoptozy. Podobnych obserwacji dokonano w embriogenicznych kulturach *Picea abies*. Zasadne byłoby ponowne zweryfikowanie twierdzenia Haberlandta, że u *Oenothera* śmierć komórek spowodowana nacięciem w sąsiedztwie zalążka może prowadzić do partenogenezy, co zresztą było kwestionowane przez późniejszych eksperymentatorów.

SŁOWA KLUCZOWE: nekrohormony, embriogeneza.