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Response Letter

Autophagy :Executioner of programmed cell death in plants?

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We are grateful for the interest that Cacas and Diamond have shown towards our Trends in Plant Science opinion article on the timing and co-ordinate regulation of plant cell death[1]. They identified a potential catachresis in our definition of autophagic programmed cell death, whereby they suggest that the autophagy observed in fatally committed cells may not be directly inducing cell death. Our initial definition of "autophagic cell death" was more intended to highlight that autophagy was a predominant feature of this type of cell death, irrespective of its role in the death process.

Although our review article was written from a broad signalling perspective, we are glad that it generated a response from those interested in autophagy. Autophagy is a tightly controlled process, whose investigation frequently produces conflicting results. For example, knocking out genes regulating autophagy may either promote or inhibit autophagic pathways; which in turn may impetuously inhibit or promote the cell death processes. This probably reflects the complexities of potential feedback regulation and the type of studies carried out. Here, we briefly reiterate the evidence for pro-life and pro-death roles of autophagy.

Cacas and Diamond quite correctly ingeminate that autophagy has a pro-life function. This has been well characterised in a plethora of different organisms/cell types such as human haemopoietic cells [2], *C. elegans* (*Caenorhabditis elegans*) [3], *D. discoideum* (*Dictyostelium discoideum*)[4], and tobacco (*Nicotiana tabacum*)[5]. In these cases it was demonstrated that inhibition of autophagy by ablation or silencing of AUTOPHAGY (ATG) genes, led to cell death during hormone and nutrient deprivation, and pathogen attack. The pro-life function of autophagy is possibly due to its capacity to improve cellular fitness via a controlled vacuolar recycling and redistribution of nutrients. This process is known to inhibit apoptosis-like cell death in plants.

Conversely, there is also considerable evidence to suggest that autophagy may also have a "pro-death" function. Consequently, we were concerned when Cacas and Diamond stated that "there are only two examples published thus far showing that the successful execution of PCD (programmed cell death) requires functional autophagy machinery *in vivo*: the work of Berry and Baehrecke [6]on degradation of salivary gland cells in *Drosophila* (*Drosophila melanogaster*), and that of Veneault-Fourrey *et al.* [7]reporting on cell death associated with spore germination of the rice blast fungus". In fact, there have been many studies carried out on *C. elegans*[8], HeLa cells[9], mouse embryonic fibroblasts[10] and murine fibrosarcoma cells[11] in which the genetic or chemical ablation of autophagy components suppresses cell death; thereby illustrating that in many situations autophagy is a requirement of PCD. Since autophagy has a pro-death role in many different organisms, it is compelling to make the assumption that this also applies to plants; and indeed there is some evidence to support this. Knocking out *atg7* and *atg9* in *Arabidopsis* leads to either normal or enhanced levels of autophagy, with the concomitant development of premature senescence (see Cacas and Diamond). This is consistent with the idea proposed by Cacas and Diamond who stated that "elevated atg-dependent autophagy can lead to cell death". However, the mechanics behind this process are poorly understood at present.

It is possible that the outcomes of autophagy, whether it be pro-life or pro-death, is dependent on the method of influencing atg gene regulation (i.e genetic knockouts or gene silencing) and the particular treatment applied to the organism. For autophagy to be properly correlated to pro-life or death functions, it has to be measured in an appropriate manner; which is often difficult since there are many different assays, each with their own caveats. Recently a large consortium of experts published guidelines for accurately measuring autophagy, which should enable a more thorough interpretation of experimental results[12]. This coupled to the identification of mediators/regulators of autophagy and their protein interactors, should enable an elaborate delineation from molecular switch to phenotype. Such data will hopefully ameliorate any current debates and will foster the development of more advanced plant autophagic models which could be used to finely dissect pro- life and death functions.

For autophagy to participate in seemingly contrasting biological functions in plants, there must be cross-talk and possibly some level of functional redundancy in the autophagic pathways; as was implied by Cacas and Diamond. At present, these mechanisms remain elusive. We must also not just consider the down stream effects of autophagy- the upstream signals must also be considered, since they induce the process and likely help determine if pro-life or pro-death routes are taken. We touched on several of these possible modulators in our Opinion article, and look forward to understanding how they contribute to the autophagic crosstalk.

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