News & Views

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No sex please, we're (in)breeding

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A genetic trick allows induction of haploid maize plants by a process known as gynogenesis, which is a useful tool for breeders. In this issue of *The EMBO Journal*, Gilles *et al* (2017) show that loss of function of a patatin-like phospholipase A underlies the induction of gynogenesis, findings that were also made in two other recent studies (Kelliher *et al*, 2017; Liu *et al*, 2017).

See also: **L Gilles *et al***

Plant reproduction is characterized by an "alternation of generations"—the dominant diploid sporophyte phase in higher plants is punctuated by short, multicellular haploid phases in male and female gametophytes buried within the flowers. Double fertilization produces two major structures, the diploid embryo, and triploid endosperm, a nutritive tissue for the embryo. Crop breeders rely on recombination events that occur during plant sexual reproduction to shuffle alleles and optimize agronomic traits, such as yield, and resistance to stresses and pests. For some crops, however, a very significant boost can also be obtained using F1 hybrids that rely on "hybrid vigor" or heterosis to produce yields far surpassing the parental strains (Shull, 1948). The production of stable, uniform hybrids relies on the ability to make different inbred lines, which can take several years, as it requires multiple generations of self-crossing. To overcome this time constraint, maize breeders use a genetic trick discovered in the 1950's by Ed Coe at the University of Missouri. Early in his career, Ed noticed that one of his stocks (the sixth one he grew, "stock 6") produced ~2–3% of weak, runty plants when crossed as a male parent onto different female lines (Coe, 1959). He went on to demonstrate that stock 6 was giving rise to haploid progeny by a process known as gynogenesis, where the male gametes induce haploid embryo formation solely from the mother's chromosomes, and Ed's stock 6 was therefore dubbed a "haploid inducer". Normal maize lines produce haploid embryos at a much lower frequency, but use of stock 6 made it into a practical tool for breeders. Haploid maize plants are sterile, since they cannot undergo meiosis, but treatment with colchicine is used to stimulate spontaneous chromosome doubling, to allow the production of fertile, perfectly inbred lines in a single generation. This simple trick has been used widely in maize breeding, but the underlying molecular mechanism of haploid induction has remained a mystery for the past ~60 years.

In the past month, however, three groups reported the identification of the major causal gene for haploid induction in maize stock 6 (Gilles *et al*, 2017; Kelliher *et al*, 2017; Liu *et al*, 2017). Quantitative trait mapping had identified a number of loci responsible for haploid induction, and the major one, on chromosome 1, was identified using a combination of genetic fine mapping and genomic sequencing. The locus mapped to a patatin-like phospholipase gene expressed in mature pollen, and molecular complementation, RNAi knockdowns, TALEN, or CRISPR gene knockouts were used to confirm that the mutation in this gene was responsible for the haploid induction trait. This gene was named *NOT LIKE DAD* (*NLD*), *MATRILINEAL* (*MTL*), or *ZmPHOSPHOLIPASE A1* (*ZmPLA1*) by the three groups based in France, US, and China (Gilles *et al*, 2017; Kelliher *et al*, 2017; Liu *et al*, 2017). Remarkably, all haploid inducer lines appear to derive from the same original stock, as they each carried the same 4-nucleotide insertion in the last exon of the causal gene, causing a frameshift and early truncation of the encoded protein (Fig 1). Fluorescent protein fusions were used to localize NLD/MTL/ZmPLA1; however, the results were somewhat conflicting: Gilles *et al*, (2017) showed plasma membrane localization in *Arabidopsis* root cells and in maize sperm cells, suggesting that NLD/MTL/ZmPLA1 may function in signaling sperm cell or gamete fusion, whereas in Kelliher *et al*, (2017) the NLD/MTL/ZmPLA1 protein was observed in the sperm cell cytoplasm. The frameshift harboring NLD/MTL/ZmPLA1 fusion protein failed to accumulate in sperm cells in both studies (Gilles *et al*, 2017; Kelliher *et al*, 2017), while it was mis-localized to mostly cytoplasmic and endomembrane compartments when expressed in *Arabidopsis* roots (Gilles *et al*, 2017). Imaging of the wild-type GFP fusion indicated that both sperm cells were delivered into the female embryo sac (Kelliher *et al*, 2017). Despite the conflicting subcellular localization reports, the timing of NLD/MTL/ZmPLA1 expression suggests it is involved in promoting fusion of sperm and egg cells, and/or in suppression of embryo formation by the unfertilized egg. Whatever the mechanism, timing appears to be critical during haploid induction, since fertilization of the central cell by one sperm cell occurs normally to produce endosperm, but the other sperm cell likely does not fuse with the egg cell. Alternatively, fertilization of the egg cell might occur normally, followed by post-zygotic elimination of paternal chromosomes (Ishii *et al*, 2016). More careful time-lapse imaging of the NLD/MTL/ZmPLA1 fluorescent protein fusions should help resolve these hypotheses.

Molecularly, there also remains much to learn. The inducer gene encodes a predicted phospholipase A, an enzyme that cleaves phospholipids, and functions in membrane remodeling as well as in formation of

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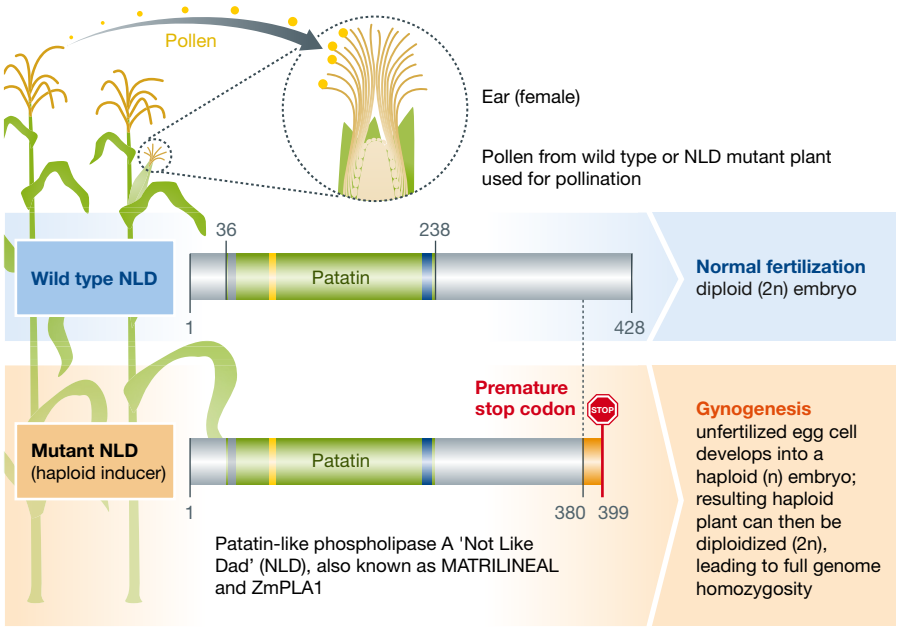

Figure 1. Role of the NLD gene in maize fertilization.
A frameshift mutation in the maize NLD gene leads to the generation of haploid maize plants in a process known as gynogenesis.

lipid-derived signaling molecules. The NLD/MTL/ZmPLA1 protein has phospholipase activity *in vitro* (Kelliher *et al*, 2017), but other than that, its actual mode of action remains somewhat of a mystery. mRNAseq analysis gave some insights, comparing gene expression in pollen of haploid inducer versus matched non-inducer lines. Several pollen-specific genes were found to be mis-expressed in haploid inducer pollen, including genes involved in transporter functions, endomembrane trafficking, or signaling. Also genes involved in Ca^{2+} signaling known to be involved in fertilization in plants and animals (Dresselhaus *et al*, 2016; Kelliher *et al*, 2017) were mis-expressed. While these genes provide some interesting leads, clearly much remains to be discovered in the mechanism of this fascinating aspect of plant reproduction.

In summary, the discovery of the haploid inducer gene from stock 6 brings some closure to a > 60-year mystery in maize biology and breeding. Excitingly, identification of the haploid inducer locus means this procedure should be readily transferable by genome editing to other crops, including rice where hybrids are already being applied to increase yields (Huang *et al*, 2016). Together with additional strategies for haploid induction, including engineering of CENTROMERIC HISTONE3 (Ravi & Chan, 2010), these strategies will hopefully enable breeders to sustainably feed a growing planet, where yield increases of ~70% are projected to be needed by 2050 to feed more than 9 billion mouths (Ray *et al*, 2013; http://www.fao.org/news/story/en/item/35571/icode/). These discoveries could also benefit developing country agriculture, since the CIMMYT Global Maize Program is already developing tropically adapted inducer lines (Prigge *et al*, 2012). Gynogenesis also occurs in some animals and is an important tool in commercial fish breeding (Komen & Thorgaard, 2007), and it will be fascinating to know whether similar fertilization mechanisms are conserved across kingdoms.

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