NAD$^+$ accumulation as a metabolic off switch for orthodox pollen

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Terrestrial plant pollen is classified into two categories based on its metabolic status: pollen with low-metabolism are termed “orthodox” and pollen with high-metabolism are termed “recalcitrant.” Nicotinamide adenine dinucleotide (NAD) is crucial for a number of metabolisms in all extant organisms. It has recently been shown that NAD homeostasis plays an important role in a broad range of developmental processes and responses to environment. Recently, a reverse genetic approach shed light on the significance of NAD biosynthesis on pollen fate. In orthodox Arabidopsis pollen, NAD$^+$ that was accumulated in excess at dispersal dramatically decreased on rehydration. The lack of a key gene that is involved in NAD biosynthesis compromised the excess accumulation. Moreover, absence of the excess accumulation phenocopied the so-called recalcitrant pollen, as demonstrated by the germination inside anthers and the loss of desiccation tolerance. Upon rehydration, NAD$^+$-consuming inhibitors impaired tube germination. Taken together, our results suggest that accumulation of NAD$^+$ functions as a physiochemical molecular switch for suspended metabolism and that the decrease of NAD$^+$ plays a very important role during transitions in metabolic states. Shifting of the redox state to an oxidizing environment may efficiently control the comprehensive metabolic network underlying the onset of pollen germination.

Terrestrial plants are equipped with mechanisms to maintain internal stability in the face of developmental and environmental changes. “Developmental arrest” is one of the strategies for adaptation to adverse environmental challenges.1 One exemplary phenomenon is programmed desiccation (i.e., drying without dying) that occurs during male gametophyte development in almost all extant gymnosperms and a large proportion of angiosperms; the resultant pollen with low-moisture and low-metabolism are termed “orthodox.”2 They remain viable for a few days or longer.3 Maintenance of orthodox pollen enables plants to avoid futile energy cycling, thereby allowing them to survive under air-dry conditions in nature until the pollen acquire water from the stigma, at which point they immediately begin germinating. Therefore, the metabolic status of pollen can play an important role in cell longevity. In contrast, in some angiosperms, the pollen does not undergo this desiccation process and contains relatively higher moisture levels as well as higher metabolism. Such pollen, termed “recalcitrant,” are metabolically active even after dispersal from the anther. Though the recalcitrant pollen can survive for a few hours only due to high susceptibility to desiccation damage, they can immediately elongate the pollen tube and execute subsequent fertilization (as observed in cleistogamous flowers). In other words, cross-pollination with the recalcitrant pollen can be limited to very proximate female sporophytes of chasmogamous flowers. Thus, a better understanding of the mechanisms underlying the physiological properties that allow classification of pollen into the categories “orthodox” and “recalcitrant,” based on the status of metabolism and desiccation is fairly

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significant in the evolution and establishment of reproductive isolation in plants.

The molecular mechanisms underlying the desiccation of orthodox pollen are thought to be analogous to those of seeds because seeds are another example of a reproductive structure demonstrating developmental arrest. For example, many studies have reported on intracellular physical properties, antioxidative scavengers and accumulation of protective molecules or compatible solutes. In contrast, no molecular genetic studies have been published that explain how the orthodox pollen maintain low metabolic rate without dying. As a result of failure in orthodox pollen, the rate of desiccation tolerance is probably due to the loss of the functional role and biological implications of NAD metabolism and the loss of desiccation tolerance are significant with regard to pollen desiccation susceptibility. Orthodox pollen may

The involvement of pyridine nucleotides in a broad range of processes including bolting, senescence, and abiotic stress adaptation suggests that not only the nicotinamide adenine dinucleotide (NAD) pools but also the NAD metabolism regulator(s) may play important roles in these processes. We have used reverse genetics to identify the functional role of NAD metabolism during microspore development. We found that orthodox Arabidopsis pollen accumulates oxidized NAD (NAD$^+$), resulting in a much lower redox state at dispersal. It was observed that hydration drastically decreased the NAD$^+$ level and consequently increased the redox state. NAD$^+$-consuming enzyme inhibitors efficiently retarded germination, and excess NAD$^+$ loading impaired normal pollen tube growth. On the basis of these results, we speculate that NAD$^+$ accumulation regulates pollen fate, and acts as a negative regulator of pollen germination (Fig. 1). Based on our hypothesis, nmnat pollen lacking NAD$^+$ accumulation during microspore development enables them to germinate and the tube to elongate inside the anther under high-humidity conditions, thus mimicking rtl pollen and typical recalcitrant pollen. Therefore, NAD$^+$ appears to participate directly in the molecular regulation of germination onset, although NAD$^+$ accumulation is not essential for adequate pollen germination (Fig. 1). This mode of action of NAD$^+$ was also supported by the fact that dispersed nmnat pollen was round, resembling hydrated pollen, the ectopic callose deposition and shortened pollen longevity observed under air-dry conditions are probably due to the loss of desiccation tolerance. The occurrence of germination inside the anther and the loss of desiccation tolerance are conclusive evidences to prove that the dispersed nmnat pollen remain metabolically active. A feasible mechanism, by which NAD$^+$ accumulation downregulates pollen metabolism, could be the impairment of NADH-dependent redox reaction, which is essential for tube germination; for example, downregulation of mitochondrial ATP synthesis and reactive oxygen species (ROS) generation, by shifting the redox state to a more oxidizing environment. Taken together, we hypothesized that accumulation of NAD$^+$ functions as a physiochemical molecular switch of suspended metabolism, and that decrease of NAD$^+$ plays a crucial role during metabolic state transitions (Fig. 1).

Our results indicate that the NAD-associated redox homeostasis may be significant with regard to pollen desiccation susceptibility. Orthodox pollen may
invest substrates (e.g., Asp) and energy (e.g., ATP) for NAD biosynthesis to control the redox balance during microspore development to acquire desiccation tolerance, thereby expanding its outcrossing distance for heterogamy in a fluctuating environment. Because orthodoxy and recalcitrance are not strict categories, dispersible distance and germination timing will vary depending on both the redox state at anthesis and the potency of NAD decrease at rehydration. It is noteworthy that mnnat is, at least, non-allelic to rgl and dissimilar to rgl-like, suggesting the existence of other factors controlling the metabolic switching off associated with the regulatory mechanisms of NAD biosynthesis or unknown downstream pathways. Screening of components that are involved in tuning the redox state suitable for pollen metabolism in response to rehydration is now underway at our laboratory.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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