GRAS Genes and the Symbiotic Green Revolution

Michael K. Udvardi and Wolf-Rüdiger Scheible

Industrial nitrogen fixation, which annually converts 100 million metric tons of N₂ to NH₃, produced fertilizer that fueled the agricultural Green Revolution (1). A different Green Revolution occurred 65 million years earlier when some plants discovered a way to utilize atmospheric N₂ directly, through symbiosis with nitrogen-fixing bacteria. Evolution of symbiotic nitrogen fixation won these plants a competitive edge in natural environments with limited mineral- and organic-nitrogen. Nitrogen fixation by bacteria, called rhizobia, within legume plants now annually injects about 50 million metric tons of nitrogen into agricultural systems (2) in an environmentally friendly manner. The rhizobia reside in nodules that develop on the roots or stems of legumes in response to signaling between plant and bacteria. Both organisms reprogram their gene expression and metabolism in response to the interaction (3). Genetic analyses have revealed some of the plant genes and proteins essential for nodule development and function (3–5). In this issue, Kaló et al. on page 1786 (6) and Smit et al. on page 1789 (7) describe two putative transcription factors of the GRAS family that appear to link the initial rhizobial signaling molecule, Nod factor (modified oligosaccharide), to changes in plant gene expression that are required for nodule development (6, 7). GRAS proteins are a recently discovered family of plant-specific proteins named after the three founding members: GAI, RGA, and SCR. Recruitment of the two legume GRAS proteins into the symbiotic program may have been key to the evolutionary success of this family of plants.

The recent adoption of *Medicago truncatula*, a relative of alfalfa, and *Lotus japonicus* as models for legume research has greatly accelerated the genetic mapping and isolation of genes required for nitrogen fixation, especially those involved in rhizobial-plant signaling and nodule development (3, 4). Among the signaling components discovered in these two species are probable components of the Nod factor receptor (8–10); a leucine-rich repeat receptor–like kinase (11, 12); a pair of putative plastid cation channels (13, 14); and a calcium/calmodulin-dependent protein kinase (CCaMK) (15, 16), which may decode a calcium oscillation signal (17), and cellular processes (20). Second, GRAS proteins possess several structural features typical of transcriptional regulators, although direct DNA binding has not yet been shown. Third, both Nsp1 and Nsp2 are nuclear proteins. Each protein, when fused with green fluorescent protein (GFP) and expressed in plant root cells (6, 7), translocated to the nucleus, where it rescued the nodulation defects of nsp1 or nsp2. Although Nsp1 is constitutively expressed in the nucleus (7), Nsp2 relo-
Perspectives

cates from the nuclear envelope to the nuclear lumen in response to Nod factor detection by the cell (6). However, the NSP2-GFP localization studies were done with a gene construct driven by the strong constitutively active cauliflower mosaic virus (CaMV) 35S promoter, which the authors admit can lead to spurious localization results, so care must be exercised in interpreting the Nod factor–dependent relocation of the NSP2 fluorescence fusion protein. Expression of NSP1-GFP in transgenic roots was driven by the native nspl promoter, which avoids this concern. The nuclear location of NSP1 and NSP2 is interesting, not only because this puts both proteins within easy reach of genes, but also because CcAMK, which acts upstream of NSP1 and NSP2 in the Nod factor signaling pathway (18), is located in the nucleus as well (6, 7). Indeed, CcAMK may physically interact with either NSP1 or NSP2 (or both). Phosphorylation of the NSP proteins by this kinase may activate them, promoting transcription of target genes. The calcium-spiking signal that has been observed in cytoplasm following Nod factor perception (4) may propagate into the nucleus to be interpreted by CcAMK.

A portion of the rhizobial signaling pathway in legumes does double duty. The components from downstream of the Nod factor receptor to just upstream of the NSP proteins (see the figure) are also used by mycorrhizal fungi to establish beneficial symbioses with legumes. Mycorrhizal symbioses, which provide plants with phosphorus and other nutrients, evolved ~460 million years ago when plants first colonized the land (21). In fact, such symbioses may have been a prerequisite for successful colonization. Mycorrhizal symbioses still exist in 90% of land plant species. Ancestral legumes may have coopted part of the signaling machinery of this ancient symbiosis to facilitate the more recent symbiosis with nitrogen-fixing rhizobia (21). NSP1 and NSP2 are not part of this shared portion of the signaling pathway; instead they act later to confer specificity, which enables the plant to respond appropriately to rhizobial infection alone.

Another transcription factor implicated in legume nodule development, NIN from L. japonicus (22), probably acts downstream of NSP1 and NSP2 and the early transcriptional responses in root cells. NSP1 and NSP2, however, are key links in the early Nod factor signaling chain, and their discovery will lead to a better understanding of the molecular interactions that make the pathway functional.

With this identification of NSP1 and NSP2, most of the proteins that are indispensable for early rhizobia-legume signaling are now known. However, many questions about the detailed workings of this pathway remain. Does CcAMK interact with and phosphorylate either NSP1 or NSP2? Do NSP1 and NSP2 interact physically? Do NSP1 or NSP2 bind directly to the promoters of genes that are activated by Nod factor signaling? Do NSP1 and NSP2 act cooperatively on the same target genes? The plethora of questions surrounding these and other Nod factor signaling components presages exciting times in symbiosis research.

References

Archaeology

Glassmaking in Bronze-Age Egypt

Caroline M. Jackson

Ever since Sir Flinders Petrie discovered evidence for Bronze-Age glass production in Tell el-Amarna, Egypt, in the late 19th century (1), controversy has surrounded his findings. Does the evidence represent primary glass production (raw materials were mixed to produce glass) or secondary working (ready-made glass was imported and reworked into artifacts)? The answer has important implications for understanding trade and exchange in the Mediterranean during the late second millennium B.C. On page 1756 of this issue, Rehren and Pusch (2) provide evidence in favor of primary production in Egypt.

In the Late Bronze Age, glass was a high-status commodity. Any group that controlled its production or consumption would have occupied a powerful position. Archaeological evidence of a rise in trade and consumption indicates that this was a period of political change throughout the Near and Middle East and the Mediterranean area. This transformation may be explained by the rise of elite groups who chose to express allegiances through competitive gift exchange of prestigious artifacts. Glass—being difficult to work, complicated to produce, and available in vivid, symbolically significant colors—was favored for use in such artifacts. Understanding the evidence from Amarna will help to define the role of prestige goods and how elites used them to enhance their position.

The first glass vessels found in Egypt were stylistically indistinguishable from the earlier Mesopotamian glasses. The only contemporary written accounts of glasses are from the Amarna tablets. These small, sun-dried clay tablets document dispatches to and from the Egyptian courts and, in the case of glass, record a request by the pharaoh Akhenaten (~14th century B.C.) for glass to be brought to Egypt. These strands of evidence suggest that glass was not produced in Egypt, but only reworked there.

However, stylistic analysis and analysis of textual accounts are not the only ways to understand the trade in, and manufacture of, glass. The composition of a glass will vary when different raw materials and recipes are used, in principle allowing both technology and provenance to be investigated with chemical fingerprinting. Egyptian glasses were produced from silica (probably from quartz pebbles) and a soda-rich plant ash flux, which should vary in composition depending on where the raw materials were procured. Therefore, glasses with similar compositions would suggest that they were produced with similar raw materials and technology and were made at the same production center. Ideally, we may expect to see different chemical fingerprints of glasses made at different factories, or at least differentiate glasses with respect