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Genetic modification, intercellular communication, and epigenetic regulation in plants: An outlook

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Photosynthetic plants have a special place in living nature as they generate unbelievable amounts of carbohydrates, i.e., ca. 150×10^{12} kg on a global scale per year, and they are responsible for the production of the molecular oxygen in the Earth's atmosphere. Thus, historically, studies of plants have been associated in the minds of scientists and laypeople alike with agriculture and related biotechnology fields. However, it is very far from reality. Already decades ago, plants joined the ranks of other higher model organisms, such as insects and animals, both from the standpoint of conceptual and technical sophistication of experimental approaches as well as their influence on our advances in the knowledge of biology.

Unlike most other organisms, plants as individuals have no capacity for travel and thus are unable to escape their surroundings. Along the eons of their evolution therefore plants have accumulated many biological capabilities to adapt to the changing biotic and abiotic environments. For example, plants can alter the degree of lipid unsaturation in their cellular membranes, and, therefore, alter the membrane fluidity, depending on the ambient temperature, i.e., increase it in the cold and decrease it in the heat [1]. Or, plants, in which the outside access to the cell membrane is limited due to the cell walls that encase the individual cells, have evolved intercellular connections, termed plasmodesmata [2], which are gateable and allow cell-to-cell transport of macromolecules and macromolecular structures, from nucleic acids to proteins to subviral particles [3–6]. Whereas the plasmodesmata were discovered more than 120 years ago [7], the concept of macromolecule/particle transport through intercellular connections emerged only 20 years ago with the discovery of membrane tunneling nanotubes (TNTs) [8], and since then TNTs have been shown to traffic large macromolecules and viruses [9–14], translating the concept developed in pants to the animal organisms. Even the discovery of viruses and, consequently, the science of virology began with the Tobacco mosaic virus (TMV), a plant RNA virus [15,16]. This cell-to-cell movement of plant viruses through plasmodesmata was one of the two biological (and, at the time, largely enigmatic) processes that attracted me to plant biology. Another process historically studied in plant systems is genetic engineering.

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Declaration of competing interest

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In nature, diverse species of plants are genetically modified by a bacterial pathogen *Agrobacterium tumefaciens* which transfers to plants and integrates into their genome a segment of its plasmid DNA, termed T-DNA; the genes contained in the T-DNA induce neoplastic cell growth as well as biosynthesis of opines, amino acid derivatives that are secreted by the tumor cells into the environment and are used by Agrobacterium as carbon and nitrogen sources. In the laboratory, the native T-DNA is re- placed by the sequences of interest and used to produce transgenic plants [17–22]. Like Agrobacterium, many human pathogenic bacteria, e.g., Bartonella, Legionella, Helicobacter, and Shigella, have evolved to export their virulence effector proteins into the host cells using the type 4 secretion system (T4SS) machinery [23], and, at least one of these bacterial species, *Bartonella henselae*, was reported to export and integrate into a human cell genome a plasmid reporter DNA [24,25], although it remains unknown whether Bartonella exports its own, endogenous DNA and genetically transform its human host cells *in vivo*, during the course of infection.

The field of plant biology continues to provide excitement and intellectual and technical challenges to all involved. This is a very broad field of science that encompasses all aspects of living nature as they occur in plants. I am especially interested in three molecular aspects of the plant life cycle—genetic modification, intercellular communication, and epigenetic regulation—which include the transport of biologically active nucleic acids, i.e., transgenes and viral genomes, and transcriptional regulation of their expression (Fig. 1). In these areas, as in many others in plant research, I look forward to the increased involvement of synthetic biology. Generally, synthetic biology principles and techniques allow manipulation and refactoring, i.e., replacement of endogenous regulation of natural gene circuits with synthetic, orthogonal regulatory elements, many aspects of eukaryotic or prokaryotic cells, including orthogonal control of gene expression, genetic toggle switches, and logic gates as means to control gene expression with precision, and computationally designed control of cell and tissue-specific expression [26–29]. For example, orthogonal systems, i.e., engineered biologically active molecules that cooperate to provide a specific biological function without affecting or being affected by the corresponding endogenous cellular systems [27,28], would allow the conversion of living cells with particular natural capabilities into nanomachines dedicated solely to that specific set of capabilities, e.g., refactoring Agrobacterium into a dedicated genetic transformation nanomachine. Because Agrobacterium can genetically modify a wide range of eukaryotic cells, from plant to yeast to human [19], under laboratory conditions, the refactored Agrobacterium cells could be adapted to specific target cells/organisms, facilitating their genetic modification for medical, research, and biotechnological purposes. Furthermore, the optimal molecular composition of the refactored Agrobacterium would also shed new light on the cellular mechanisms involved in the genetic transformation process.

Agrobacterium preferentially integrates its T-DNA into the double-stranded DNA breaks (DSBs) in the plant genome, presumably using the cellular DNA repair mechanisms [30–33]. Thus, our investigation of plant genetic transformation by Agrobacterium represents an aspect of the broader field of studies of the plant DNA damage response pathways. Collectively, future advances in these studies should facilitate elucidation of perhaps the most important of the remaining enigmatic steps of the Agrobacterium-mediated genetic transformation: the identity of the cellular proteins and the bacterial effectors that

participate in T-DNA integration, the molecular interactions between these factors, and the temporal sequence of these interactions that culminates with the integration event. Similarly, I anticipate important advances in our understanding of the molecular reactions and sequential steps of targeting protein and nucleoprotein cargos to plasmodesmata, increase in plasmodesmal permeability, transit of the plasmodesmal channel by the cargo molecules, and the energy sources for this active transport. Detailed proteomic characterization of plasmodesmata, which has already begun [34–36], will facilitate the mechanistic studies by identifying and characterizing the complement of proteins associated with plasmodesmata. For example, by analogy to the dynamic composition of the nuclear pore complex [37], plasmodesmal proteins that are early in plasmodesmata assembly and stable in their residence time are likely structural in function whereas those that are late in the assembly and transient in the residence time are likely directly involved in the cell-to-cell transport process.

Both plant genetic transformation and, in many cases, plasmodesmal transport of viral and endogenous nucleoprotein complexes ultimately lead up to the expression of the transferred nucleic acid molecules, i.e., transgenes, viral genomic DNA or RNA, or non-cell-autonomous transcripts. One major factor in regulating the expression and/or formation of these molecules is the posttranslational modification of histones, which determines the active or inactive state of the chromatin. These modifications, which together determine the transcriptional outcomes [38,39], are dynamic, effected by writers and erasers, i.e., histone modifying enzymes that add or remove the specific functional groups [40]. In plant cells, one major class of important, yet relatively sparsely characterized, erasers are histone deubiquitinases that have been implicated in diverse aspects of physiology, growth, and development [41–47]. Only about 10% of all 50 deubiquitinases encoded by the Arabidopsis genome [48] have been proposed to target histones and participate in epigenetic regulation [46,47,49–56]. Of these, two histone deubiquitinases, UBP26 and OTLD1, participate both in transcriptional repression and activation of their target gene expression [46,47,51,53,54], with the dual function of OTLD1 likely to be direct [46,47]. Future understanding of how deubiquitylation of the same type of histone by the same histone deubiquitinase can elicit two opposing effects on transcription of the direct target genes may help will define a potential junction in chromatin remodeling pathways leading to transcriptional repression or activation.

This short Commentary reflects my own specific and relatively narrow scientific interests. Yet, it illustrates how plant-centered model systems contribute to our understanding of diverse and fundamental aspects of life.

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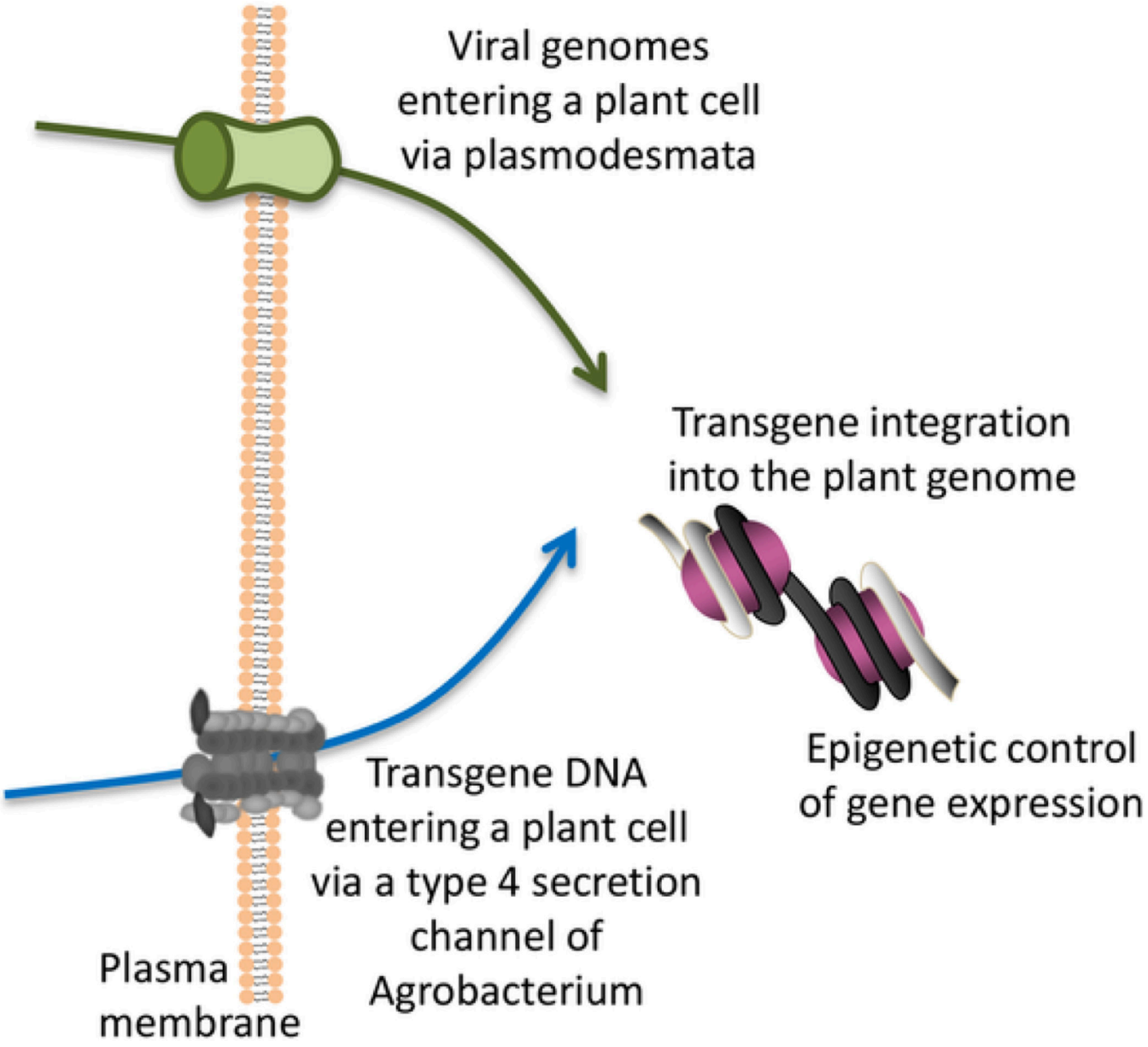

Fig. 1.

Schematic illustration of three biological processes—plant genetic engineering, plant virus cell-to-cell movement, and epigenetic regulation of plant gene expression—which involve the transmembrane transport of biologically active nucleic acids molecules and represent the focus of this Commentary.