Commentary

Bacterial RNA – a new MAMP on the block?

To defend themselves against pathogens, plants – like other organisms – need to discriminate 'self' from 'non-self' and 'modified/damaged self'. Conserved microbial molecules, so-called pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs) are key indicators of 'non-self' (Sanabria *et al.*, 2010), while the occurrence of plant-derived molecules that are normally absent (so-called danger-associated molecular patterns or DAMPs) can be a sign of 'damaged self' (Heil & Land, 2014). Both types of molecules are typically released in the context of microbial colonization and thus serve as alarm signals that consequently induce the activation of plant defense responses. In this issue of *New Phytologist*, Lee *et al.* (pp. 785–797) provide experimental evidence that 'non-self' bacterial RNA can serve as a trigger of innate immunity in *Arabidopsis thaliana* and thus functions as a PAMP/MAMP (hereafter referred to as MAMP) in plants.

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MAMP and DAMP perception

Plants are able to sense MAMPs and DAMPs with the help of dedicated cell surface receptors that are also termed pattern recognition receptors (PRRs). These are typically plasma membrane-resident receptor-like kinases that are composed of an extracellular domain for the perception of a MAMP, a single transmembrane domain for membrane anchorage, and a cytoplasmic kinase domain for intracellular signal transduction (Zipfel, 2014). Activation of such receptors, which often function in association with a co-receptor, initiates a number of canonical cellular responses that are seemingly crucial for the execution of appropriate defense measures and/or alerting neighboring cells. These prototypical cellular responses comprise, amongst others, the generation of reactive oxygen species (ROS), the execution of mitogen-activated protein kinases (MAPKs), the turning on of defense gene expression and the local formation of deposits composed of the polyglycan callose (Zhang & Zhou, 2010). In metazoans (multicellular animals) the activation of innate immunity is conceptually equivalent and likewise involves the perception of MAMPs via PRRs (Nürnberger *et al.*, 2004; see also later).

Over the past two decades, a number of plant-active MAMPs and DAMPs have been identified and in part thoroughly characterized. Typical MAMPs that are recognized by plants comprise, for example, microbial cell wall components (e.g. bacterial peptidoglycan and lipopolysaccharide, fungal chitin and oomycete β-glycans) and abundant proteins of microbial origin (e.g. bacterial flagellin and elongation factor EF-Tu; Newman *et al.*, 2013). Extracellular ATP and particular peptides represent instances of plant-active DAMPs (Heil & Land, 2014). For some of these MAMPs and DAMPs, the cognate cell surface receptors have been identified and functionally studied (Zipfel, 2014).

Nucleic acids as MAMPs and DAMPs

Heterologous nucleic acids (i.e. certain types of DNA and RNA) represent MAMPs that can be perceived by metazoan cells via certain members of a class of membrane-resident receptors known as Toll-like receptors (TLRs). The human genome encodes 10 different TLRs, which generally function as PRRs in innate immunity by sensing different MAMPs (Takeda & Akira, 2005). Regarding the detection of foreign nucleic acids, it is well established that TLR3 recognizes double-stranded RNA, an indicator of viral infection; TLR7 and TLR8 identify single-stranded RNA in endosomes (likewise indicative of viral infection); and TLR9 is a receptor for unmethylated CpG sequences in DNA molecules, which rarely occur in eukaryotic DNA. In addition to their role in recognizing single-stranded RNA of viral origin, TLR7 and TLR8 were recently identified as the human receptors for bacterial RNA (Eberle *et al.*, 2009; Eigenbrod *et al.*, 2015). In mice, a different TLR, TLR13, is responsible for the detection of bacterial RNA (Hidmark *et al.*, 2012). Bacterial 23S ribosomal RNA (rRNA) has been identified as the molecule recognized by this TLR (Oldenburg *et al.*, 2012). To date neither DNA nor RNA has been reported as a MAMP that can be detected by plants. However, fragmented extracellular 'self DNA' was recently described as a potentially novel plant DAMP of possible ecophysiological relevance (Duran-Flores & Heil, 2015; Mazzoleni *et al.*, 2015a,b; Veresoglou *et al.*, 2015).

Bacterial RNA – a new plant-active MAMP?

In their present study, Lee *et al.* (2015) observed that leaf infiltration of plants with total RNA from bacteria (*Pseudomonas syringae* pv. tomato DC3000, 'non-self RNA'), but not with Arabidopsis RNA ('self RNA'), results in induced innate immunity, exemplified by dose-dependent growth inhibition of

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P. syringae pv. tomato DC3000 bacteria in systemic leaves. This effect is seemingly attributable to intact or specifically processed bacterial ribonucleic acids, since neither sheared (randomly fragmented) RNA nor RNase-treated RNA provoked such growth inhibition. Moreover, potentially contaminating DNA or proteins could be ruled out as the causal agents, because bacterial DNA was inactive as a trigger of plant defense, while proteinase K-treated RNA retained its capacity to reduce bacterial propagation.

The authors went on to demonstrate that total bacterial RNA can induce characteristic MAMP responses. They discovered that the bacterial RNAs cause the generation of superoxide anions, but not hydrogen peroxide (H_2O_2). They also trigger MAPK activation, defense gene expression and the formation of callose deposits. Interestingly, intracellular signaling pathways that rely on the defense-associated phytohormones salicylic acid (SA) and jasmonic acid (JA) seem to be involved in the transduction of the RNA-triggered signal. Plants that are defective in either SA- or JA-mediated signaling were compromised in their ability to reduce bacterial growth upon pretreatment with bacterial RNA. Mutant analysis further suggested that the well-characterized Arabidopsis PRRs FLS2, EFR and CERK1 are not responsible for the perception of bacterial RNA.

Since rRNA is a major constituent of total RNA preparations, Lee *et al.* considered the rRNA portion as the MAMP-active principle of total bacterial RNAs. A crudely enriched rRNA fraction indeed largely recapitulated the effects of total bacterial RNAs with regard to the generation of superoxide anions, defense gene expression and enhanced immunity. The latter phenotype could also be achieved upon pretreatment with *in vitro*-synthesized bacterial rRNA, which further strengthens the notion that rRNA might be the MAMP-active bacterial determinant.

In sum, the authors provide several lines of evidence that bacterial RNA functions as an elicitor of innate immunity in *Arabidopsis thaliana*, thereby claiming a novel plant-active bacterial MAMP. Given that 'non-self' types of nucleic acids have been long known as inducers of innate immunity in metazoans, this finding seems not entirely surprising, yet remarkable. It is astonishing that such an obvious MAMP candidate molecule escaped the attention of, and discovery by, the plant community for such a long time. It could be the notorious sensitivity of RNA to rapid degradation that might have precluded the belief that this type of nucleic acid could act as a genuine MAMP in plants. It is indeed difficult to envisage how such a labile biopolymer, possibly released by ruptured bacteria (Fig. 1), could persist at sufficient concentrations for a long enough time in the coarse conditions of the apoplast to induce plant immunity. The plant extracellular space harbors constitutive levels of secreted ribonucleases (MacIntosh *et al.*, 2010); thus the half-life of any RNA molecule in this environment might be comparatively short. At present it remains unknown whether the RNA concentrations used in the experiments by Lee *et al.* (150 ng μl^{-1}) come close to any physiologically relevant concentrations, and which fraction of the RNA in fact reaches the plant plasma membrane, the site where presumptive MAMP receptors are supposedly located. Besides potential degradation by apoplastic ribonucleases, the cell wall permeability of macromolecules such as RNA has to be considered in this respect.

Is ribosomal RNA the actual elicitor?

One may further speculate about the MAMP-active signatures of bacterial RNAs. The fact that sheared or even enzymatically digested total RNA is inactive with respect to the induction of immunity largely rules out the possibility that single nucleotides could be the actual trigger. Nevertheless, it would be informative to test the *dorn1* mutant, which is defective in the perception of extracellular ATP (Choi *et al.*, 2014), for its ability to induce innate immune responses upon treatment with bacterial RNA. Preliminary evidence provided by the authors suggests that the

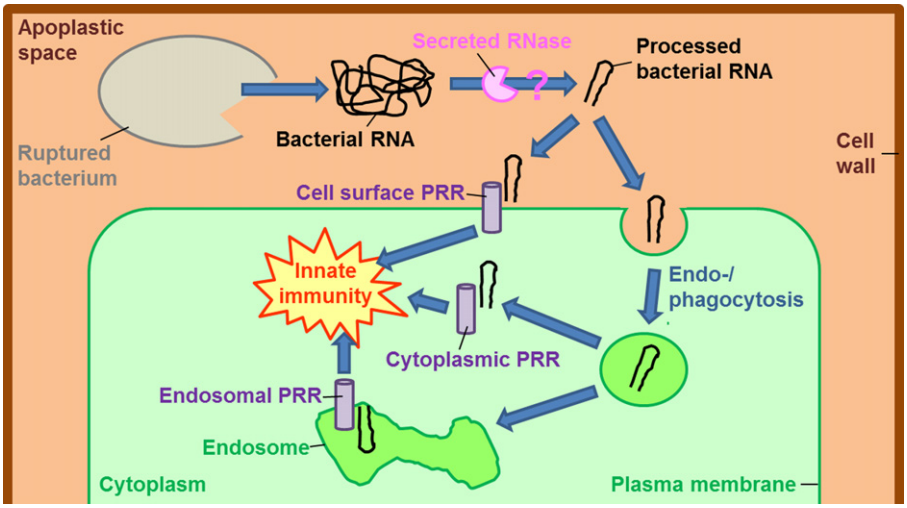

Fig. 1 Proposed perception of bacterial RNA via plant pattern recognition receptors (PRRs). The graphic depicts a scheme of a plant cell. Bacterial RNA is liberated from a ruptured bacterium and possibly fragmented by a secreted plant RNase. The processed RNA can be either directly perceived by a cell surface PRR or taken up by the plant cell via endo-/phagocytosis. Intracellularly, perception may either occur by endosomal PRRs or subsequent to the release of the RNA to the cytoplasm by cytoplasmic PRRs. Activation of PRRs finally leads to innate immunity.

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rRNA fraction might be the active principle (Fig. 1). However, the rRNA isolation method performed in the study was rather crude (excision of rRNA bands from agarose gels), and the *in vitro*-synthesized rRNA was not deployed in all types of experiments. It would be interesting to know whether *in vitro*-synthesized bacterial rRNA can also trigger the full set of canonical MAMP responses observed with the total bacterial RNA preparation. Ribosomal RNA as the elicitor of innate immunity would be analogous to the situation in mice, where a portion of the bacterial 23S rRNA has been found to cause the activation of innate immunity via the TLR13 receptor (Hidmark *et al.*, 2012; Oldenburg *et al.*, 2012). The comparatively low levels of nucleoside modifications in bacterial rRNAs is key for the activation of human TLRs (Karikó *et al.*, 2005; Rimbach *et al.*, 2015); this feature could likewise be the one that is crucial for the activation of the plant innate immune system. A particularly rigid domain of a possibly enzymatically processed rRNA molecule, probably rich in robust secondary structures, could explain its supposed stability in the apoplastic environment (Fig. 1).

The presumptive RNA receptor: the hunt is on!

With regard to the presumptive receptor of bacterial RNAs, cell surface PRRs are naturally the first candidates to think of. This type of receptor would perceive the RNA molecule on the extracellular side (Fig. 1). However, some of the RNA-triggered responses observed by Lee *et al.*, such as the lack of H_2O_2 production, the moderate activation of MAPKs and the involvement of SA- and JA-dependent signaling pathways, do not follow the canonical scheme for known plant PRRs. One might therefore hypothesize that the RNA receptor might be a protein that is not a classical plasma membrane-localized PRR. An alternative scenario would be the endocytic (or phagocytic) uptake of bacterial RNAs and their perception by endosomal receptors. Such a situation would be analogous to the localization of the human TLR7 and TLR8 receptors, which in fact reside in endosomal compartments (Takeda & Akira, 2005). Finally, potential cytoplasmic receptors have to be taken into account (Fig. 1). The present study by Lee *et al.* delivers in any way enough food for thought, and it surely spurs a plethora of ideas for future experiments.

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