Macromolecule exchange in *Cuscuta*-host plant interactions

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Abstract

Cuscuta species (dodders) are parasitic plants that are able to grow on many different host plants and can be destructive to crops. The connections between *Cuscuta* and its hosts allow movement of not only water and small nutrients, but also macromolecules including mRNA, proteins and viruses. Recent studies show that RNAs move bidirectionally between hosts and parasites and involve a large number of different genes. Although the function of mobile mRNAs has not been demonstrated in this system, small RNAs are also transmitted and a silencing construct expressed in hosts is able to affect expression of the target gene in the parasite. High throughput sequencing of host-parasite associations has the potential to greatly accelerate understanding of this remarkable interaction.

Introduction

Parasitic plants of the genus *Cuscuta* (dodders) are extraordinary examples of plant evolution in terms of their interactions with other plants. About 200 *Cuscuta* species are distributed throughout the world and they are destructive to trees, shrubs and cultivated crops such as tomato, cranberry, alfalfa, etc. [1,2]. They are members of the family Convolvulaceae and evolved from autotrophic ancestors, but specialized to live parasitically by connecting to host plants in order to extract water, nutrients, and metabolites. *Cuscuta* species are holoparasitic on their hosts and although some species synthesize chlorophyll, others have lost extensive portions of their plastic genomes so as to be functionally nonphotosynthetic [3]. The plants are unusual in many other ways as well. Shortly after attachment to a host the parasite root senesces and *Cuscuta* draws all subsequent water from the host. *Cuscuta* also appears to lack leaves, which are reduced to vestigial scales, and as a result the vegetative structure of the plant consists of stem, ranging in color from greenish to pale yellow or orange, depending on the species, which coils around their hosts and connects to host tissues via specialized organs termed haustoria.

Given their obligate dependence on the host, *Cuscuta* are highly attuned to finding and parasitizing their hosts. *Cuscuta* seeds germinate in the spring like other annual plants, but here the similarities end. The *Cuscuta* shoot is a thread-like stem that grows upward, rotating in a counterclockwise direction until contacting a vertical host stem. Location of the host is accomplished with sophisticated mechanisms including detection of light quality, with blue and far red wavelengths promoting coiling and haustoria formation [4]. Volatile chemical signals emitted by hosts and non-host plants also aid in host detection by attracting or repelling the parasite such that the odds of contacting a favored host species are improved [5]. Another essential signal is touch, and as the parasite encounters a host stem it coils around it and forms haustoria along the inner side of the coil (Figure 1). The haustoria penetrate the host to form vascular connections from which the parasite obtains all of its resources. The parasite shoot then grows, branching prolifically and forming new connections to create a network of stems connected to hosts, and potentially bridging several different host species simultaneously.

Various aspects of *Cuscuta* biology have been reviewed in recent years, including overall parasitic mechanisms [2,4], host resistance [6] and transfer of materials [7,8]. This review will focus on the most recent advances in understanding host-*Cuscuta* interactions with particular emphasis on haustorial transfer of macromolecules that may act in information exchange.

***Cuscuta*-host connections: a unique system**

Perhaps the most unique aspect of the *Cuscuta* connection to its host is the open translocation of substances between the two organisms. In addition to small molecules such as water and photosynthates, *Cuscuta* takes up macromolecules including nucleic acids, and even pathogens such as viruses and phytoplasmas (Table 1). Although many aspects of this interaction have been studied, precise mechanisms regulating macromolecule transmission remain unclear.

The *Cuscuta* haustorium initiates in the parasite stem from cortical and epidermal cells that expand to form a lateral protuberance, variously termed the upper haustorium, adhesive disk or pseudohaustorium, which elongates toward the host stem and adheres by means of excreted pectinaceous “cement” material [9,10]. From this develops the lower haustorium, which invades the host and then gives rise to searching hyphae, specialized cells that elongate between and through host cells to reach the host vascular tissue (Figure 1D)[11]. The ability of searching hyphae to grow through host cells creates a situation in which host cell walls form in association with new parasite cell walls and are spanned by plasmodesmata (Figure 1F). These plasmodesmata represent clear points of symplastic connection between the species and offer a potential route for exchange of macromolecules. In addition, the searching hyphae connect to host vascular tissue, and those that contact host xylem differentiate into xylem cells, whereas those that contact host phloem differentiate into phloem-type cells with aspects of transfer cells (Figure 1G,H)[12]. The xylem connections form an open conduit between host and parasite, whereas the phloem connections between host and parasite involve membrane and cell wall barriers. Nevertheless, movement of labeled sugars, phloem-mobile dye and virus strongly indicate symplastic continuity [13].

RNA trafficking in the host-*Cuscuta* interaction

RNAs have roles beyond coding for protein, including acting at multiple levels to regulate gene expression. Within a single plant, mRNAs may move between cells to transmit information over short or long distances, and this may be a mechanism for coordinating growth and development [14,15]. The finding that hosts and *Cuscuta* exchange RNA raises the possibility that this type of communication can occur in an interspecies manner, such that mRNA movement is not even limited to the organism in which it was produced [16,17].

The scope of RNA exchange between host and *Cuscuta* can be large and bidirectional. A study of *Cuscuta pentagona* growing on *Arabidopsis* estimated that about 1% of the RNAs in the parasite stem near the point of haustorial attachment to the host were of *Arabidopsis* origin, while in the reverse direction 0.6% of RNAs in the *Arabidopsis* stem were of *Cuscuta* origin [18**]. The diversity of genes represented among these mobile RNAs was high, as 44% (>9,000) of expressed host transcripts were detected as mobile

into the parasite and 24% of expressed *Cuscuta* unigenes were detected in the host. A similar experiment using *C. reflexa* identified over 2,000 mobile transcripts from *Arabidopsis* [19**]. It is noteworthy that the magnitude of RNA movement depended on the host species used in the interaction (greater exchange with *Arabidopsis* vs. tomato hosts) and likely also depends on the *Cuscuta* species, experimental conditions, depth of sequencing, etc. [18**,19**]. But together these studies indicate that substantial proportions of host and parasite transcriptomes are capable of intercellular mobility.

Mobility of such diverse transcripts raises the question of how RNA moves between the species and the extent to which this movement is regulated. Patterns of movement suggest that some regulation occurs, but no specific features of transcript form or function were correlated with mobility. One major predictor of mobility is simply the abundance of a transcript in the region of the haustorial connection, but this alone cannot account for mobility as evidenced by contrasting examples of highly expressed non-mobile and lowly expressed mobile transcripts [18**,19**]. A study of mRNA movement across root-shoot graft junctions in *Arabidopsis* provides compelling evidence that transcript mobility is regulated by factors such as nutrient status and that transcripts may move in directional patterns (e.g. root to shoot and *vice versa*) and be targeted to specific organs [19**]. With this in mind it is interesting to consider movement of *Cuscuta* transcripts into the host. Such flow against the predominant source-sink gradient is consistent with bidirectional transport in phloem. *Cuscuta* mRNAs moving into host cells would be advantageous for the parasite if they facilitate communication and colonization of host tissues.

The exact cellular route of RNA exchange is uncertain, but is consistent with parasite connections to host parenchyma and phloem (Figure 1F and H). *In situ* hybridizations of mRNAs moving from host to *Cuscuta* indicated that host mRNA transfer occurred between host parenchyma cells and searching hyphae of the parasite [17], ultimately translocating long distance in *Cuscuta* phloem [17,20]. No size cutoff for RNA transfer was identified for *Cuscuta* and its *Arabidopsis* or tomato hosts [18**], although the mass limit for protein movement was determined to be between 27 and 36 kDa [21]. Under normal circumstances RNA movement between plant cells is thought to be mediated by RNA association with proteins that facilitate passage through plasmodesmata [22]. The exchange of RNA between hosts and *Cuscuta* appears to be closer to a “bulk flow” model of mobility, and it is possible that the pathogenic nature of *Cuscuta* parasitism creates abnormally open connections with hosts.

The fate of host transcripts in the parasite is not clear. Possibilities include translation to protein in the recipient organism, processing into silencing signals that can affect gene expression, or simply degradation into component nucleotides. A tomato transcript

moving into *Cuscuta* largely disappeared within 8 hrs of the parasite stem being severed from the host [20]. While evidence of mRNA function is currently lacking, a mobile protein has shown evidence of function. The herbicide metabolizing enzyme phosphinothricin acetyl transferase (PAT) was detected in *Cuscuta* growing on PAT expressing transgenic soybean and appeared to protect the parasite against glufosinate injury [23*].

Silencing RNA

The best evidence that mobile RNA has an effect on gene expression of the recipient plant comes from trans-specific gene silencing experiments. This approach uses a gene sequence specific to the parasite and expresses it in the host in a hairpin construct to generate a mobile silencing signal that affects the target gene in the parasite. The clearest examples of this to date in parasitic plants come from *Triphysaria versicolor*, a root parasite in the family Orobanchaceae, in which siRNAs expressed in the host reduce expression of a gene expressed in the parasite [24,25*]. For *Cuscuta* this technique was employed to silence *SHOOT MERISTEMLESS-Like*, a gene encoding a parasite transcription factor important in shoot development, resulting in malformed haustorial searching hyphae and reduced parasite vigor [26*]. This approach has potential to be a breakthrough technology in the control of parasitic weeds, at least for those species that absorb the siRNA signals from their hosts [27-29]. As a research tool it is currently the best approach for gene function studies for *Cuscuta*, which has not been successfully transformed and regenerated. The significance of small RNA movement between host and parasite species is not known. However, to the extent that small RNAs are an important component of plant genome regulation [30], it is likely that the parasite would have a mechanism for using both self and non-self RNAs. An intriguing possibility is that *Cuscuta* siRNAs move into the host and act as effectors to manipulate host defenses similar to the way the fungus *Botrytis cinerea* suppresses host immunity [31].

Parasite transcriptomes aid in understanding host interactions

Transcriptome sequencing using high throughput technology has great potential for studying host-parasite interactions. Given that the parasites and their hosts are different species and often phylogenetically distant from each other, it is possible to sequence RNA from intermixed tissues at the haustoria-host connection and then separate the two transcriptomes based on prior knowledge of host genomic sequences. As more parasite transcriptomes and genomes become available this process will increase in ease and accuracy. This approach is being used to identify genes that are important in haustorial development and provide insight into parasite-host interactions.

Transcriptional analyses of *Cuscuta* at various stages of development identified genes associated with the haustorium of *Cuscuta* [32**]. Genes specifically induced during haustorial stages include those involved in cell wall modification (e.g., pectin methylesterase, cellulase, and expansins), which is a recurring theme in parasitism [18**,33,34] and consistent with the needs of the parasite to deform host cells and expand its own cells during haustorial invasion. Other genes induced include those related to pathogen or stress response (indicating a role for plant defense as a component of parasitism) and increased transporter activity (consistent with the uptake of nutrients). Shoot tip regions of *C. pentagona* and *C. suaveloens* were enriched in transcripts encoding proteins involved in biological adhesion, which may aid in forming connections to new hosts [35]. Considering the broad host range of *Cuscuta*, it would be interesting to know whether haustorial connections differ depending on the host contacted. This is true for *Triphysaria* haustoria, which induced expression of specific expansin genes appropriately depending on whether it was parasitizing monocot or dicot hosts [33].

Horizontal gene transfer (HGT)

HGT comprises another type of macromolecule exchange and one that is especially prevalent between parasitic plants and their hosts [36,37] (See also the review in this issue [38]). Although no fitness benefit has been proven for HGT-acquired genes, *Cuscuta* and *Phelipanche* species both obtained an apparently functional *albumin 1* gene from legumes [39] and strictosidine synthase-like gene from *Brassicaceae* [40], suggesting that these HGT events provide competitive advantages. Considering the constant exchange of mRNA in *Cuscuta*-host interactions described above, it is interesting that examples of RNA-mediated HGT have not been reported. The closest example is a nuclear encoded gene that appears to have moved from *Sorghum* into *Striga hermonthica* and contained potential remnants of a poly-A tail [41]. Rather, the mechanism of HGT between plants is likely mediated through genomic DNA transfer [37,42,43]. The physical contact between host and parasite may lead to the same type of cell-cell contact that has been reported in DNA or organelle exchange in plant graft interactions [44,45]. No systemic movement of DNA has been documented in parasite-host interactions.

Conclusions

Cuscuta has many characteristics of an ideal subject for studying plant-plant interactions. Starting as a seedling it exhibits sophisticated mechanisms for locating a host, integrating into host tissues, and extracting host nutrients. The ability to establish symplastic connections with a wide range of host species such that it can exchange RNAs and proteins is an especially intriguing aspect of the interaction (Box 1). Given that *Cuscuta* has evolved to mix its RNAs with those of a host, it is reasonable to

assume that it also has mechanisms to deal with this added complexity. Perhaps *Cuscuta* can distinguish self from non-self RNAs, using each type of transcript for a specific purpose. Alternatively, *Cuscuta* may have evolved to function without regard for transcript source, processing them all equally. Regardless which of these possibilities is closer to the truth, *Cuscuta* will provide valuable insights into mobile RNAs and plant interactions.

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Table 1. Summary of the types of molecules and organisms that move between hosts and *Cuscuta*.

Category	Examples	<i>Cuscuta</i> species	Host species	Reference
Water		<i>C. reflexa</i> ; <i>C. costaricensis</i>	Coleus; <i>Tithonia tubiformis</i>	[46,47]
Metabolite	Sugars, amino acids	<i>C. reflexa</i>	Lupin	[48]
Herbicide	Glyphosate and ALS inhibitors	<i>C. campestris</i>		[49]
Protein	Green fluorescent protein; Phosphinothricin acetyl transferase	<i>C. reflexa</i> ; <i>C. pentagona</i>	Tobacco; Soybean	[23*,50]
mRNA	Many	<i>C. pentagona</i> <i>C. reflexa</i>	Tomato, <i>Arabidopsis</i> , Alfalfa, Pumpkin	[16,17,18**,19**]
siRNA	<i>Shoot Meristemless-like (STM)</i>	<i>C. pentagona</i>	Tobacco	[26]
DNA	<i>atp1</i> , <i>atp6</i> , <i>matR</i> ;	<i>C. gronovii</i>	<i>Plantago</i>	[42]
Viroid	HSVd	<i>C. subinclusa</i>	Cucumber	[31,51]
Virus	PVY ^N	<i>C. reflexa</i>	Tobacco	[13]
Phytoplasma	Yellows disease	<i>C. campestris</i>	Periwinkle	[52]

Box 1. Outstanding questions in *Cuscuta*-host interactions.

1. What mechanisms govern the movement of mRNA between hosts and *Cuscuta*?
2. Do *Cuscuta* exhibit host-specific RNA exchange when parasitizing different hosts?
3. What is the fate of host mobile RNAs in the recipient organism: Translation, gene regulation, or degradation?
4. Are non-self RNAs identified and processed differently from self RNAs?
5. Are *Cuscuta* RNAs important in haustorial penetration and function in hosts?
6. Does RNA movement via *Cuscuta* contribute to horizontal gene transfer in plants?

Figure legend

Figure 1. *Cuscuta* haustorial connections with its host. **(A)** Overview of *Cuscuta* (yellow stem) growing on an *Arabidopsis thaliana* host plant. **(B)** Close-up view of *Cuscuta* coiled around host stem tissue. Swollen areas of *Cuscuta* stem are points of haustorial development. **(C)** Cross-section of a coil showing *Cuscuta* haustoria penetrating the host stem (vascular bundles indicated by blue (phloem) and red (xylem)). **(D)** Magnified view of an invading haustorium showing intrusively growing searching hyphae. **(E)** Magnified view of a mature haustorium showing the connection of parasite cells with host vascular tissues. **(F)** Further magnification of the chimeric cell wall between searching hyphae cell and host parenchyma cell, joined by interspecific plasmodesmata. **(G)** Further magnification of *Cuscuta* connection with the host xylem cells (red lines). **(H)** Further magnification of *Cuscuta* connection with the host phloem cells (blue lines). Although the exact path of mRNA exchange is unclear, evidence points to involvement of both plasmodesmatal (F) and phloem (H) connections. [Illustrations based on 11,12,21,53,54].

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Figure

Figure 1