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Genomic-scale exchange of mRNA between a parasitic plant and its hosts

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the S_4 state—the Mn^{IV}-oxyl radical and the Mn ligated μ-oxo bridge (Fig. 4) (*29*)—facilitating low-barrier O-O bond formation (*35*, *38*, *41*).

This mechanistic framework provides a blueprint for the design of synthetic water-splitting catalysts. It is an all-Mn^{IV} complex that proceeds to the O-O bond–forming transition state, with its formation coupled to the inclusion of the second substrate water at the open coordination site within the heterometallic cubane. This concerted process, oxidation coupled to substrate binding, may be of benefit in avoiding the binding/activation of both substrates in the resting state of the catalyst, "switching off" slow two-electron, catalase-like reactivity (*43*). Upon completion of the cycle, the heterometallic complex imposes the correct spin alignment for the two substrates in the transition state. This latter property can be exploited in heterogeneous Mn_2O_2Ca oxide materials that form layered cuboidal-like structures (*2*) and may explain why a heterometallic MnCa complex evolved to perform the water-splitting reaction in the first place.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
Figs. S1 to S12
Tables S1 and S2
References (*44*–*76*)

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PLANT SCIENCE

Genomic-scale exchange of mRNA between a parasitic plant and its hosts

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Movement of RNAs between cells of a single plant is well documented, but cross-species RNA transfer is largely unexplored. *Cuscuta pentagona* (dodder) is a parasitic plant that forms symplastic connections with its hosts and takes up host messenger RNAs (mRNAs). We sequenced transcriptomes of *Cuscuta* growing on *Arabidopsis* and tomato hosts to characterize mRNA transfer between species and found that mRNAs move in high numbers and in a bidirectional manner. The mobile transcripts represented thousands of different genes, and nearly half the expressed transcriptome of *Arabidopsis* was identified in *Cuscuta*. These findings demonstrate that parasitic plants can exchange large proportions of their transcriptomes with hosts, providing potential mechanisms for RNA-based interactions between species and horizontal gene transfer.

Cuscuta species (dodders) are parasitic plants that obtain water and nutrients from their plant hosts by using specialized organs termed haustoria. The haustoria of *Cuscuta* develop from the stem of the parasite, where it coils around the host, penetrating host tissues and ultimately forming vascular connections (*1*, *2*). These connections allow transfer of not only water and nutrients into the parasite but macromolecules, including mRNAs (*3*–*5*) and proteins (*6*), and even pathogens, such as viruses (*7*, *8*), viroids (*9*), and phytoplasmas (*10*). Here, we characterize the scope and directionality of mRNA movement.

mRNA trafficking between cells regulates plant development (*11*, *12*), with potential for controlling processes such as leaf shape (*13*, *14*), time of flowering (*15*), tuber formation (*16*, *17*), and root growth (*18*). Small RNAs can also act systemically to influence plant development (*19*), and a construct encoding a silencing RNA and expressed in a host plant can silence a *Cuscuta* gene (*20*). Although this last example is from an artificial construct, it supports the idea that RNA movement between separate plant individuals can function as a type of organismal communication (*21*). We used transcriptomics to investigate the RNA transfer between *Cuscuta* and its hosts.

Cuscuta species parasitize a wide range of broad-leaved plants (often simultaneously) and are destructive to crops, such as tomato (*Solanum*

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lycopersicum) (*22*). We grew *Cuscuta* on *Arabidopsis thaliana* and tomato hosts because the sequenced genomes of these species facilitates confident identification of host and parasite transcripts from mixed RNA populations. We harvested three distinct regions for each parasite-host association for analysis (Fig. 1A). The *Cuscuta* haustorium grows toward the center of the host stem and does not spread systemically inside the host (*2*), so the tissues harvested did not risk inclusion of endophytic haustorial tissue that could lead to cross-contamination of samples. Furthermore, the growth habit of *Cuscuta* allows unattached stem regions to be easily collected separate from the interface regions where haustoria bind tightly to host tissues (Fig. 1A and fig. S1).

To identify host and parasite mobile transcriptomes, we sequenced cDNA libraries derived from each of the three tissues. The rate of RNA mobility was expected to be low, so the first libraries were sequenced with a full lane of Illumina GAIIx (Illumina, Incorporated, San Diego, CA) for one run each of *Cuscuta* growing on *Arabidopsis* and tomato hosts. Second and third biological replicates of *Cuscuta* with *Arabidopsis* were sequenced with a full lane and one-sixth lane of the higher output HiSeq 2000 (Illumina, Incorporated) platform, respectively. This yielded over 1.6 billion high-quality reads, which were subjected to various controls by which we filtered out contaminating reads and poor quality reads and trimmed away adapters and primers. Reads were then identified as host, parasite, or too highly conserved to assign (fig. S2). The parasite reads were used to reconstruct a *Cuscuta* transcriptome assembly.

Reads from each library were stringently mapped to host and parasite transcriptomes to estimate RNA movement between the species. *Arabidopsis* read proportions in parasite tissue averaged 1.1% of total mapped reads across the three sequencing runs, whereas host stems contained 0.6% *Cuscuta* reads (Fig. 1B). Read mapping in the tomato-*Cuscuta* association suggested somewhat lower rates of transfer, but the pattern was similar to that of *Arabidopsis* with the exception of interface tissue, where the greater mass of the tomato stem likely resulted in a higher proportion of reads. Bidirectional mobility in transcript movement is consistent with the long-established ability of *Cuscuta* to transmit viruses between plants bridged by the parasite (*7*) and suggests that *Cuscuta* is capable of transmitting mRNAs between different plants.

Independent confirmation of mobility was shown by reverse transcription polymerase chain reaction (RT-PCR) amplification and subsequent sequencing of selected transcripts. Mobility of 24 tomato transcripts into *Cuscuta* has been documented this way (*3*, *23*), so we analyzed *Arabidopsis* transcripts moving into *Cuscuta* and *Cuscuta* transcripts moving into *Arabidopsis* and tomato hosts (fig. S3). Such confirmation is not practical for all mobile transcripts, but the output of read mapping itself produced a compelling picture of RNA transfer (Fig. 2). The read sequences and coverage from parasite stem tissue closely matched those of the interface tissue, with the exception that mobile mRNAs in the parasite occurred in fully spliced mature form; introns were only found in libraries derived from host stem or interface tissues.

The diversity of transcripts represented by the mobile reads was determined by high-stringency mapping of reads from the three species (*Arabidopsis*, tomato, and *Cuscuta*) to their combined reference sequences. The criterion for transcript mobility was set by using fragment counts where one fragment represents either a matched pair of reads or a single unpaired read. The threshold for mobile transcripts was set at a mean of four fragments per transcript because this level was found to produce positive RT-PCR confirmation (fig. S3), whereas eight fragments per transcript was considered strong evidence of mobility. The greatest number of mobile transcripts originated from *Arabidopsis* hosts, with 45% (9518) of the genes in the expressed *Arabidopsis* transcriptome found in *Cuscuta*, and most of these (5983) showed strong evidence of mobility (Table 1). In contrast, tomato hosts produced substantially fewer mobile transcripts than *Arabidopsis*, with 347 (1.6% of total expressed) detected in the parasite. Part of the difference between tomato and *Arabidopsis* transcript mobility into *Cuscuta* may be attributed to the single sample of tomato-*Cuscuta* sequenced and the lack of deep sequencing from a full lane of HiSeq 2000 data, but even allowing for these differences there appear to be differences in RNA transfer to the parasite from different host species.

With respect to movement from parasite to host, 8655 *Cuscuta* unigenes were classified as mobile into *Arabidopsis* stem, and 5973 unigenes showed strong evidence of mobility (Table 1). This is 24% of the 35,614 unigenes expressed in *Cuscuta*. Tomato host uptake of *Cuscuta* transcripts was again lower than that of *Arabidopsis*, with 288 unigenes showing evidence of mobility. The rates of transcript movement between *Cuscuta* and the two hosts were consistent in both directions, with a much freer exchange occurring between *Cuscuta* and *Arabidopsis* than between *Cuscuta* and tomato, suggesting that mechanisms regulating haustorial selectivity may be host-specific.

We asked whether mobile and nonmobile RNAs have distinctive properties that provide insight into mechanisms of mobility. One characteristic common to mobile transcripts was high abundance, as measured by fragments per kilobase per million mapped reads (FPKM) in the interface region, and this was especially pronounced for the *Arabidopsis* interaction with *Cuscuta* (Fig. 3A). FPKM (*24*) was used because it normalizes fragment counts to transcript length and depth of transcriptome sequencing to better estimate transcript

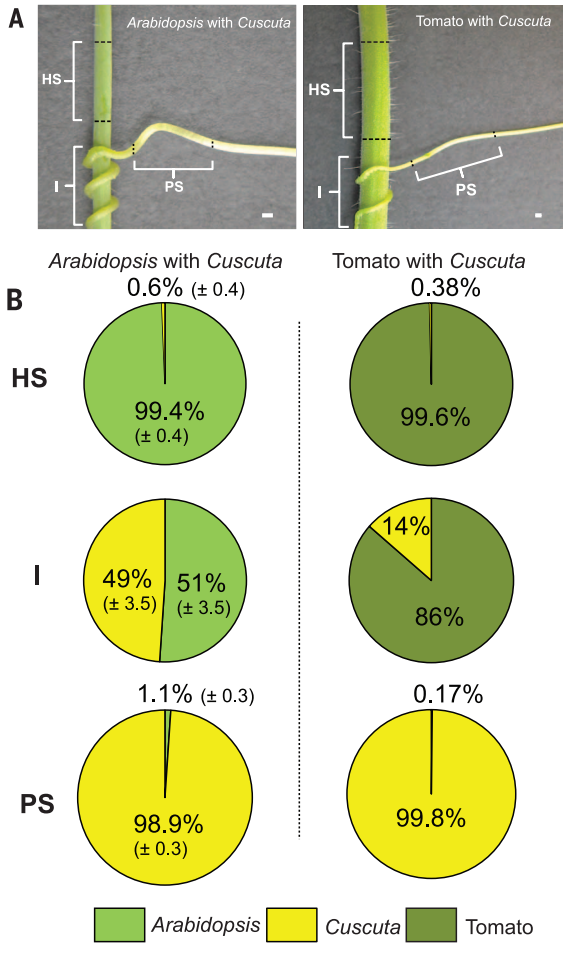

Fig. 1. Transcriptome compositions of host and parasite tissues at and near the region of haustorial attachment. (**A**) Tissues analyzed were the host stem above the region of attachment (HS), interface region where parasite is connected to the host (I), and the parasite stem near the region of attachment (PS). Scale bars represent 1 mm. (**B**) Pie charts show the proportions of reads mapped to host and parasite transcriptomes in each tissue. *Arabidopsis* with *Cuscuta* data are means (±SE) of three separate sequencing runs; tomato with *Cuscuta* data are from one run.

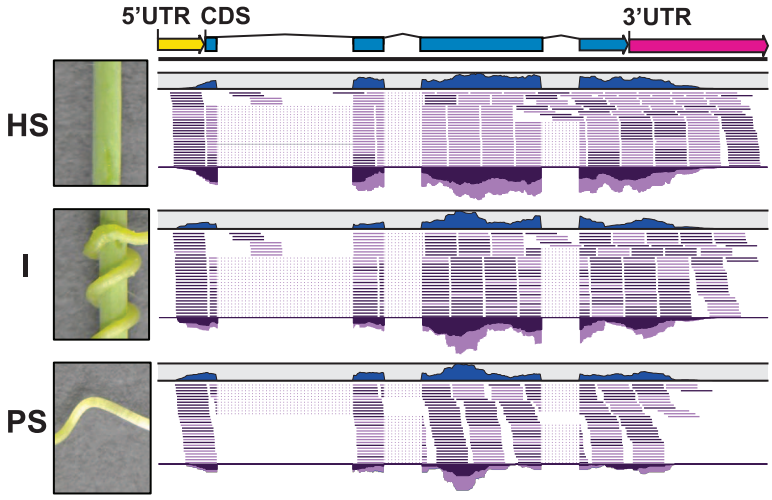

Fig. 2. Example of read assemblies of an *Arabidopsis* gene, *TRANSLATIONALLY CONTROLLED TUMOR PROTEIN* (*AtTCTP*), in host stem (HS), interface (I), and parasite stem (PS) tissues. Intron sequences were not found in sequences derived from parasite tissue. The gene model at top indicates coding sequence as blue bars and introns as line bridges. Dark and light purple lines indicate forward and reverse paired reads, respectively, as mapped to the gene model. UTR, untranslated region; CDS, coding sequence.

levels. The patterns were similar for mobile and nonmobile transcripts in the tomato-*Cuscuta* interaction, although tomato nonmobile transcripts spanned the spectrum from low to high abundance (Fig. 3B). This indicates that one aspect of transcript mobility is related to their high abundance in the cells near the host-parasite boundary, but it is not the only factor influencing mobility, as evidenced by the many transcripts with similar expression levels yet differing mobility.

To consider whether transcript mobility is associated with gene function, we used OrthoMCL software to generate orthologous clusters of mobile and nonmobile gene classes that were common to all three species (*25*) (Fig. 3C). Assigning genes from these clusters to gene ontology terms led to the identification of terms enriched among mobile and nonmobile classes (table S2). Restricting the list to those terms that were only enriched for multiple species (e.g., transcripts from both *Arabidopsis* and *Cuscuta*) yielded smaller sets of terms that may reflect core mobile and nonmobile categories (Fig. 3D). These results demonstrate that mobility can be correlated with gene function, but the mechanistic basis for such correlations remains obscure. For example, a large proportion of mobile transcripts are assigned to the response-to-stimulus term; it is possible that these transcripts are specifically targeted for intercellular mobility, but it is also possible that characteristics of transcript accumulation or localization in the cytoplasm makes them especially prone to host-parasite exchange.

Further evidence for selective mobility of RNAs comes from plots of transcript abundance in the interface region versus abundance of the same transcripts in the parasite stem (Fig. 4). The plot of *Arabidopsis* to *Cuscuta* mobile transcripts showed that levels of most transcripts in the parasite were about one-hundredth of those in the interface tissues, indicating that most transcripts follow the same dynamics of movement (Fig. 4A). However, some host RNAs appear to move more readily into the parasite and occurred at FPKM levels in the parasite nearly equal to those in the interface (seen as outliers above the main group in Fig. 4). The tomato-*Cuscuta* data showed a more-dispersed pattern of mobilities that supports the idea that dynamics of movement differ between tomato and *Arabidopsis* hosts (Fig. 4B).

Table 1. Numbers of genes and unigenes with mobile transcripts from hosts into *Cuscuta* or *Cuscuta* into hosts. The numbers represent transcript reference (the *Arabidopsis* Information Resource reference annotation TAIR 10/International Tomato Annotation Group annotation ITAG 2.4/*Cuscuta* unigenes) sequences as categorized by number of fragments detected in self and nonself tissues. A threshold of four fragments per gene was used to determine transcript detection, with four fragments detected in nonself tissues considered evidence for mobility and eight fragments providing strong evidence for mobility.

Mobility category	*Arabidopsis-Cuscuta*		*Tomato-Cuscuta*	
	Arabidopsis genes	*Cuscuta* unigenes	Tomato genes	*Cuscuta* unigenes
Total mobile	9,518	8,655	347	288
Mobile (>8 fragments)	5,983	5,973	147	116
Mobile (>4 fragments)	3,535	2,682	200	172
Nonmobile	11,874	26,960	21,848	26,717
Total expressed genes/unigenes	21,392	35,614	22,194	27,005

An unresolved question regarding *Cuscuta* haustoria is the precise route used to acquire material from the host. Substantial physiological evidence points to symplastic connections consistent with direct transfer between phloem tissues of host and parasite [e.g., (*8*)], but no open phloem connections have been observed (*26*). Rather, *Cuscuta* haustorial cells share plasmodesmata with hosts across chimeric cell walls (*2*, *8*), and these have been implicated in host-parasite mobility of RNA (*4*). The long-distance movement of RNAs in the parasite suggest phloem involvement (*4*, *5*), but our data indicate that the situation is complex. We compared transcripts moving from *Arabidopsis* into *Cuscuta* to published phloem transcriptome data from *Arabidopsis* and four other species (*27*–*31*), finding significant associations between the data sets (table S3). Further analysis using the subset of *Arabidopsis* transcripts with especially high mobility into *Cuscuta* (i.e., those significantly above the mass of data points in Fig. 4A) indicated correlations with the more-robust data sets (*Arabidopsis* and ash) but did not demonstrate a linkage between phloem-associated transcripts and high mobility into *Cuscuta* (table S4). Our data also indicate that *Cuscuta* acquires transcripts such as the ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) small subunit, which is not considered part of an authentic phloem transcriptome (*32*). Taken together, these data suggest that host-parasite RNA exchange includes RNAs known to occur in phloem but also many RNAs from other cells.

We can only speculate about the importance of large-scale mRNA movement between individuals of different species. For example, some specific mRNAs transmit information long distances in plants (*13*, *14*, *18*, *33*), and these same information molecules could help the parasite track host physiological status or, in the other direction, use its own mRNA to manipulate the host to facilitate parasitism. However, it is not known whether mobile mRNAs act through translation into protein or through another mechanism, so it is unclear whether mRNAs could even function across widely different species. Host mRNAs disappear within several hours inside *Cuscuta* (*5*), but this could be due to processes such as translation into protein or degradation for nucleotide recycling. In this regard, the question of whether *Cuscuta* can distinguish its own transcripts from those of its hosts is interesting.

This widespread exchange of mRNA raises the possibility of horizontal gene transfer (HGT).

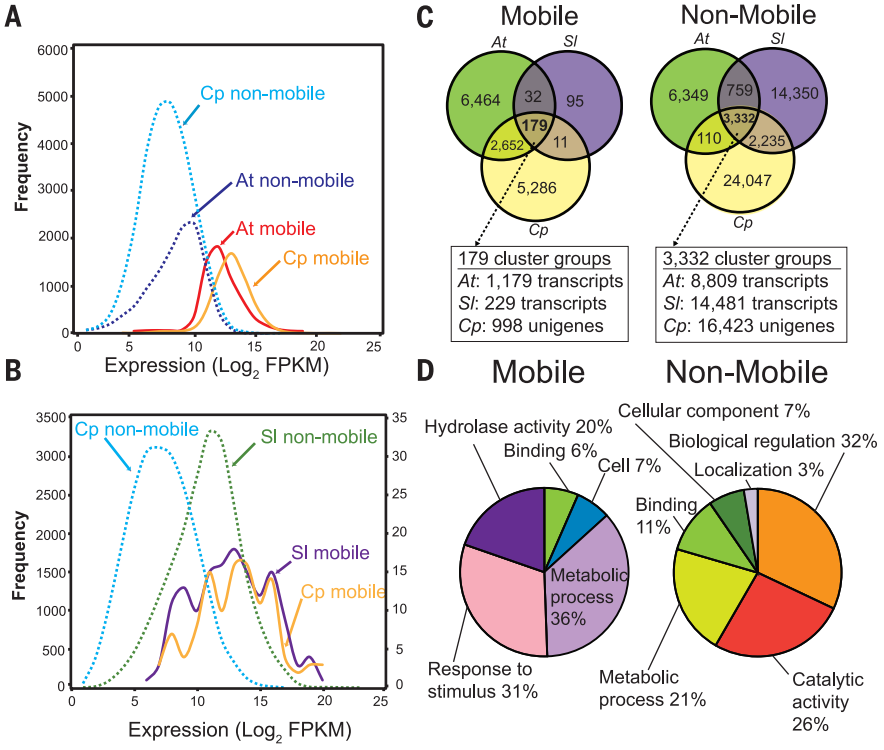

Fig. 3. Properties of mobile and nonmobile RNAs. (**A**) Distribution of transcript expression levels in interface tissue as related to mobility in *Arabidopsis-Cuscuta* associations. (**B**) Same as (A), but for mobility in tomato-*Cuscuta* associations. (**C**) Venn diagrams showing common sets of transcripts that were either mobile or nonmobile out of *Arabidopsis*, tomato, or *Cuscuta*. Numbers are orthologous clusters as determined by OrthoMCL. (**D**) Pie charts showing Gene Ontology (GO) slim terms as proportions of sets of 11 mobile and 23 nonmobile GO terms that were enriched for multiple species. The full lists of GO slim terms for these data sets and all terms significantly overrepresented and underrepresented in each of the three species are given in table S2.

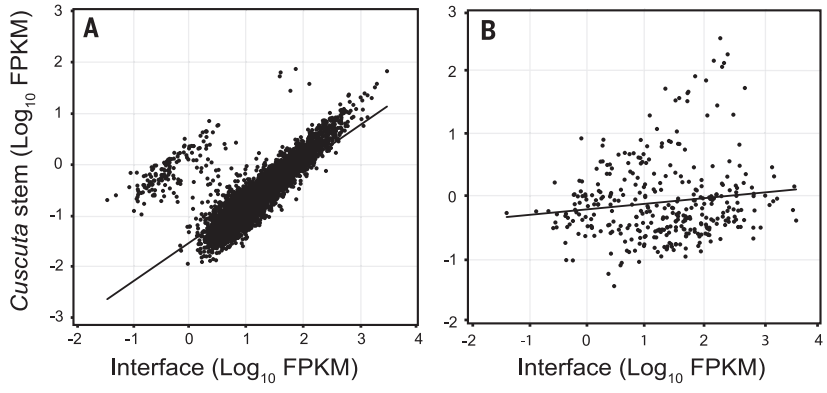

Fig. 4. Scatter plots of transcript abundance (FPKM) in parasite stem versus host-parasite interface. (**A**) A total of 9518 *Arabidopsis* transcripts identified as mobile into *Cuscuta* (Table 1). (**B**) A total of 347 tomato transcripts identified as mobile into *Cuscuta*. Lines are linear regressions of the data.

Given what appears to be a constant exchange of mRNA between *Cuscuta* and its hosts, the relative prevalence of cases of HGT involving *Cuscuta* is not surprising (*34*–*38*). Although most documented cases of HGT in parasitic plants suggest a mechanism involving direct transfer of DNA, at least one case of HGT into a parasitic plant (*Striga hermonthica*) exhibits evidence of an RNA intermediate in the mechanism (*39*). The ability of one *Cuscuta* plant to bridge many different host individuals raises the possibility that this parasite could mediate RNA exchange across different individuals and even across hosts of different species.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6198/808/suppl/DC1
Materials and Methods
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