Is There Social RNA?

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ulations, both Neandertal and Denisovan, by modern humans has been put forward to account for discordancy in tool traditions from archaeological research at sites in central India (*13*), where microblade technology had been in use only since 45,000 years ago, as compared to sites in Africa, China, and Malaysia. In these latter locations, the hominin populations appeared to be culturally modern (in terms of the tools they were making) and well adapted to the emerging localities, suggesting that the time frames implied by a short Y chronology allows insufficient time for invasion and settlement. More broadly, marine isotope 5–calibrated dates in the range of 85,000 to 130,000 yr B.P. suggest that modern humans were on the move between tropical and subtropical zones during the periods when climate oscillated in the temperate regions they would later successfully reinvade, leaving us as their legacy.

References

1. P. Francalacci *et al.*, *Science* **341**, 565 (2013).
2. G. David Poznik *et al.*, *Science* **341**, 562 (2013).
3. W. Wei *et al.*, *Genome Res.* **23**, 388 (2013).
4. R. L. Cann, M. Stoneking, A. C. Wilson, *Nature* **325,** 31 (1987).
5. E. A. Foster *et al.*, *Nature* **396**, 27 (1998).
6. H. Niederstätter *et al.*, *Forensic Sci. Int. Genet.* 10.1016/j.fsigen.2013.05.010 (2013).
7. E. Corona *et al.*, *PLoS Genet.* **9**, e1003447 (2013).
8. E. Bonilla-Silva, *Racism Without Racists: Color-Blind Racism and the Persistence of Racial Inequality in the United States* (Rowman and Littlefield, Lanham, MD 2006)
9. A. Albrechtsen, F. C. Nielsen, R. Nielsen, *Mol. Biol. Evol.* **27**, 2534 (2010).
10. R. Klein, *The Human Career: Human Biological and Cultural Origins,* (Univ. of Chicago Press, Chicago, ed.3., 2009)
11. F. Cruciani *et al.*, *Am. J. Hum. Genet.* **88**, 814 (2011).
12. A. Olivieri *et al.*, *Science* **314**, 1767 (2006).
13. S. Mishra, N. Chauhan, A. Singhvi, *PLoS ONE* **8**, e69280 (2013).

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MOLECULAR BIOLOGY

Is There Social RNA?

The idea that RNA can be transferred between organisms and function in communication and environmental sensing is discussed.

Peter Sarkies and Eric A. Miska

Our understanding of the forms, functions, and movement of RNA continues to expand. Not only can RNA control gene expression by multiple mechanisms within a cell, it appears to travel outside the cell within an organism as well. This raises the interesting question of whether the RNA world extends beyond the boundaries of the organism. Can RNA traffic integrate an organism into its environment—is there "social RNA"? Examining the mechanism of RNA interference (RNAi) may be a good route for seeking the answer.

In many eukaryotic cells, exposure to double-stranded RNA (dsRNA) can initiate an RNAi response that generates small interfering RNA (siRNA). These are potent silencing molecules that use base-pairing to recognize genes with sequence similarity to the original double-stranded trigger (*1*, *2*). Moreover, many organisms, including mustard cress and roundworms, possess mechanisms to move siRNAs between tissues (*3*, *4*). So far, research into the functions of RNAi has focused on its role within an organism—in antiviral defense or in silencing repetitive DNA sequences in the genome, for example. In the model nematode *Caenorhabditis elegans*, however, molecular mechanisms facilitate trafficking of functional RNA to and from cells. This extends the RNAi response outside of the cell and possibly even outside of the organism. The functional importance of either for *C. elegans* in the wild is still unknown. However, successfully investigating such roles could be achieved by analyzing nematodes in their natural habitat, for which ecological characterization is more advanced than for the laboratory workhorse *C. elegans*.

One of the most remarkable features of RNAi in *C. elegans* is that feeding these animals dsRNA can silence endogenous genes. This response differs from nonspecific inflammatory responses to dsRNA in mammalian cells because only genes with matching sequence to the ingested dsRNA will be silenced (*5*, *6*). A specific pathway that takes up long dsRNA (~200 to 500 base pairs) from the gut lumen involves the channel protein SID-2 (*7*, *8*). Inside the cell, dsRNA is cleaved by the endoribonuclease Dicer (DCR-1) to generate ~23-nucleotide RNAs, known as siRNAs. siRNAs are bound by Argonaute proteins, and the resulting complex targets messenger RNA for degradation. In addition, RNA-dependent RNA polymerase enzymes amplify the trigger, thereby bolstering its silencing effect on target genes (*9*). Another dsRNA-selective channel, SID-1, subsequently allows the silencing RNA signal to spread throughout the animal (*10*). The signal can even reach the germ line, thus instigating a transgenerational response (*11*–*13*).

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Podcast interview with author Eric Miska (http://scim.ag/ed_6145).

Exploiting the SID pathway enables the function of almost any gene to be examined simply by making a bacterial strain expressing dsRNA that matches the gene of interest—a great tool. But the broad implications of the response that this pathway elicits have not resonated widely, in part because its function in the normal *C. elegans* life cycle is mysterious. What possible use could there be for a pathway that takes up RNA from the environment and uses it to silence endogenous genes?

An attractively simple idea is that *C. elegans* might respond to dsRNA that is naturally produced by the bacteria it consumes. This would allow *C. elegans* to mount an RNAi response against bacterial RNAs that enter the gut. siRNAs produced by cleavage of a bacterial dsRNA trigger could target endogenous genes, redirecting gene expression programs in response to different diets. Although a plausible model, there is no clear evidence yet that this occurs. A noncoding RNA produced by certain *Escherichia coli* strains might cause gene expression changes via RNAi in *C. elegans* (*14*). However, mutations in SID pathway genes do not obviously compromise fitness under laboratory conditions, which suggests that "environmental RNAi" is not important for growth on *E. coli* in general. In the wild, *C. elegans* probably feeds on bacteria growing on rotting fruit (*15*) and therefore encounters multiple species of microbes, so deeper sampling of ecologically relevant bacteria might provide insight into the role of SID-encoding genes. Our understanding of the

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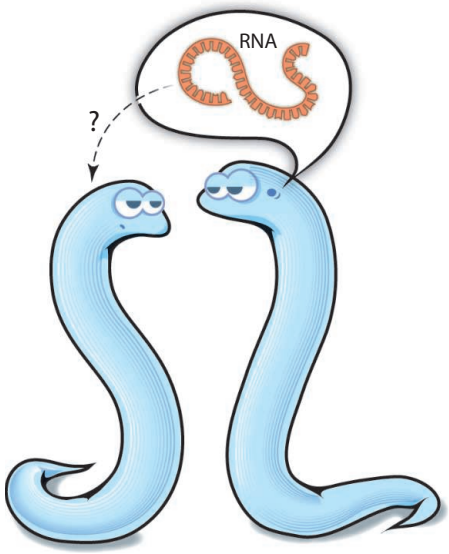
natural environment of *C. elegans* is limited, making this difficult.

An analysis of related nematodes with ecology that is better understood may provide some hints. Many parasitic nematodes undergo well-characterized life cycles involving two different hosts (*16*) (e.g., *Brugia malayi*, the causative agent of lymphatic filariasis, is transmitted between humans by mosquitoes) or both free-living and parasitic stages (e.g., the hookworm *Nippostrongylus brasiliensis* goes through two larval molts, feeding on rat fecal matter before reinfecting a rat host). RNAi has been demonstrated experimentally in parasitic nematodes of plants and animals (including human) (*17*). It is conceivable that parasitic nematodes could use host RNA as a trigger to regulate endogenous genes in response to life cycle cues.

If a parasitic strategy exists, perhaps hosts exploit it by producing dsRNA to attack parasites. Indeed, resistance to root-knot nematodes can be engineered by making transgenic plants that express dsRNA against an essential nematode gene (*18*). This points to the exciting possibility of an "RNA arms race" between host and parasite. In support of this, the genes involved in taking up RNA from the environment in *C. elegans* appear to be evolving rapidly. For example, the closely related nematode *C. briggsae* has lost the gene *sid-2* and is insensitive to feeding with dsRNA-expressing bacteria (*7*). However, expressing *C. elegans sid-2* allows *C. briggsae* to take up dsRNA from its environment. Perhaps the ancestor of *C. briggsae* was exposed to bacteria that exploited the *C. elegans* uptake pathway, resulting in selective pressure to lose the RNA uptake mechanism. But *sid-2* homologs may not be the only way by which RNA is taken up from the environment. One example is the nematode *Meloidogyne incognita,* which, despite responding to dsRNA administered through feeding, has no *sid-2* homolog. Thus, environmental RNAi is likely to occur in nematodes without obvious *sid-2* homologs (*19*).

In addition to the uptake of long dsRNA, siRNAs (downstream of DCR-1 activity) can move within *C. elegans* and can be amplified to generate abundant secondary siRNAs in the recipient tissues (*20*). This means that if the animal were exposed directly to small RNAs from its environment, these might generate an RNAi response in the absence of a dsRNA trigger. Such a process could have interesting consequences. For example, *C. elegans* could spread an antiviral RNAi response within a population to generate herd immunity. Exactly how small RNA



mobility occurs within *C. elegans*—the precise species that moves, as well as its mechanism of secretion and uptake—is unknown. When elucidated, the process should inform whether small RNAs can survive outside of the parent organism.

Small RNAs, in particular microRNAs (miRNAs), can be secreted from mammalian cells (*21*). Different body fluids, including blood, tears, and saliva, contain miRNAs, either free or contained within vesicles (exosomes) (*22*). Because miRNAs are functionally very similar to siRNAs, and indeed may have evolved from the siRNA pathway, it is plausible that comparable mechanisms of small RNA secretion might be used for siRNAs. Determining whether nematodes incorporate small RNAs into exosomes would help to clarify the range of RNA mobility, as well as the evolution of the mammalian miRNA secretion pathway.

A highly debated issue that is not yet resolved convincingly is whether secreted miRNAs from mammalian cells have an effect in recipient cells. One crucial point is that mammals do not possess amplification pathways to allow small amounts of an RNA taken up from the environment to trigger a larger response within the organism. Therefore, the miRNA itself would have to be taken up in sufficient amounts to have an effect, either with an Argonaute protein from the donor or through incorporation into the recipient cell's Argonaute complexes. Indeed, secreted miRNAs in conditioned medium from cells overexpressing miRNAs can down-regulate target genes in recipient cells. This secretion depends on GW182 proteins, which interact with Argonaute proteins (*23*). GW182 proteins could thus move with miRNAs to facilitate their effect.

There is a high degree of sequence conservation between miRNAs of different organisms. This could be important in host-parasite interactions, for example, such that miRNAs secreted from a parasite could manipulate host gene expression programs. Such cross-species traffic of miRNA has been observed. miRNAs from edible plants are found in human bodily fluids, which suggests that miRNAs might be assimilated into mammalian miRNA pathways (*24*). However, such studies suffer greatly from potential contamination artifacts and have thus proved controversial (*25*). Lab model systems such as *C. elegans* are crucial for rigorously establishing the transfer of small RNAs between organisms and for addressing mechanistic details of small RNA secretion.

Is there an extensive life for RNA molecules outside of the cell, and even outside of the organism that produced them? Investigating possible examples of "social RNA" presents an exciting avenue for future research, as the implications of such a phenomenon would be far-reaching indeed.

References

1. A. Fire *et al.*, *Nature* **391**, 806 (1998).
2. A. J. Hamilton, D. C. Baulcombe, *Science* **286**, 950 (1999).
3. C. A. Brosnan, O. Voinnet, *Curr. Opin. Plant Biol.* **14**, 580 (2011).
4. J. J. Zhuang, C. P. Hunter, *Parasitology* **139**, 560 (2012).
5. L. Timmons, A. Fire, *Nature* **395**, 854 (1998).
6. L. Timmons, D. L. Court, A. Fire, *Gene* **263**, 103 (2001).
7. W. M. Winston, M. Sutherlin, A. J. Wright, E. H. Feinberg, C. P. Hunter, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 10565 (2007).
8. D. L. McEwan, A. S. Weisman, C. P. Hunter, *Mol. Cell* **47**, 746 (2012).
9. G. Hutvagner, M. J. Simard, *Nat. Rev. Mol. Cell Biol.* **9**, 22 (2008).
10. W. M. Winston, C. Molodowitch, C. P. Hunter, *Science* **295**, 2456 (2002).
11. B. A. Buckley *et al.*, *Nature* **489**, 447 (2012).
12. A. Ashe *et al.*, *Cell* **150**, 88 (2012).
13. M. Shirayama *et al.*, *Cell* **150**, 65 (2012).
14. H. Liu *et al.*, *Nat. Commun.* **3**, 1073 (2012).
15. K. C. Kiontke *et al.*, *BMC Evol. Biol.* **11**, 339 (2011).
16. C. Dieterich, R. J. Sommer, *Trends Genet.* **25**, 203 (2009).
17. M. E. Selkirk, S. C. Huang, D. P. Knox, C. Britton, *Parasitology* **139**, 605 (2012).
18. H. M. M. Ibrahim *et al.*, *Exp. Parasitol.* **127**, 90 (2011).
19. A. G. Maule *et al.*, *Trends Parasitol.* **27**, 505 (2011).
20. A. M. Jose, G. A. Garcia, C. P. Hunter, *Nat. Struct. Mol. Biol.* **18**, 1184 (2011).
21. M.-C. Saleh *et al.*, *Nat. Cell Biol.* **8**, 793 (2006).
22. N. Kosaka, H. Iguchi, T. Ochiya, *Cancer Sci.* **101**, 2087 (2010).
23. B. Yao, L. B. La, Y.-C. Chen, L.-J. Chang, E. K. L. Chan, *EMBO Rep.* **13**, 1102 (2012).
24. L. Zhang *et al.*, *Cell Res.* **22**, 107 (2012).
25. Y. Zhang *et al.*, *BMC Genomics* **13**, 381 (2012).

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