used to rapidly probe refolding is to measure the fluorescence of tryptophan amino-acid residues in a protein. Tryptophan residues buried inside the protein fluoresce differently from those exposed to water, so that fluorescence serves as a global probe of folding. By contrast, detection techniques such as infrared spectroscopy show promise for acquiring localized structural information from proteins, and are fast enough to be combined with the laser T-jump method for initiating folding reactions.

Chen *et al.*[2] now show that mass spectrometry could join the ranks of fast, structure-sensitive techniques for studying protein folding. The first step of their technique is to add hydrogen peroxide to a cold solution of a denatured protein (Fig. 1). By using a low concentration of peroxide at low temperature, the authors ensure that the protein does not rapidly react with the oxidizing agent. Next, a nanosecond laser T-jump is used to initiate refolding of the protein. After a set time delay, the sample is then irradiated with a nanosecond ultraviolet laser pulse, which breaks up some of the peroxide into hydroxyl radicals. These radicals exist for about a microsecond and efficiently oxidize solvent-exposed protein segments, changing the protein's mass. This is the FPOP step of the process[7].

An increasingly large fraction of proteins becomes folded as time passes after the T-jump, which means that more of the amino acids become buried within the proteins' interiors. Progressively fewer amino acids are therefore exposed to solvent as folding proceeds, and so less mass is added to the protein by FPOP as the time delay between the T-jump and the FPOP step increases. By performing a series of experiments in which the time delay is varied, and then measuring the mass of the resulting protein samples using mass spectrometry, protein folding can be tracked. In practice, the protein solution flowed through the laser set-up in a capillary tube. Chen *et al.* collected the oxidized samples, quenched any remaining peroxide using a chemical scavenger, and then performed mass spectrometry on the quenched samples in a separate step.

The authors[2] used their T-jump–FPOP (TJFPOP) technique to study the refolding kinetics of denatured barstar as it adopts an intermediate conformation en route to the fully folded protein. The formation of the intermediate takes hundreds of microseconds, and had never before been observed using mass spectrometry techniques. The resulting spectra contained hundreds of peaks, and so the authors analysed only the centroid of the spectra. This simple approach limited the structural information that could be obtained, but the technique offers potentially much better time and structural resolution than was achieved in this proof-of-principle study. The ultimate time resolution of the FPOP step is about 1 microsecond, limited by the hydroxyl radicals' diffusion rate and lifetime. And as the authors point out[2], if the oxidized protein samples were enzymatically degraded before mass spectrometry[8], then analysis of the resulting fragments could pinpoint where oxidation had actually occurred. The TJFPOP technique could thus follow how amino acids in different parts of a protein become buried with time. Such a technique would be complementary to hydrogen-exchange mass-spectrometry methods, which track the formation of secondary structures in proteins by measuring how easily protons (H^+ ions) are exchanged between water and amino acids in the proteins[9].

The TJFPOP approach has some drawbacks in its current implementation. The method requires a T-jump for every kinetic data point, whereas fluorescence and infrared-detection methods collect data continuously after a single T-jump. The TJFPOP method thus introduces additional noise compared with the other techniques, and requires larger amounts of sample. Chen and colleagues' approach is also less suitable for studies at high temperatures, because the radical precursor (hydrogen peroxide) would itself react with the protein sample. But precursors more benign than peroxide can be developed.

On the plus side, a strong advantage of TJFPOP is that the whole experiment could easily be automated. But the real key to the utility of TJFPOP will be the development of suitable data-analysis techniques, to deconvolute the statistical mass distributions observed in the spectra and to analyse oxidation patterns of fragments. If these hurdles can be overcome, then the technique could provide truly massive amounts of detail about fast protein folding. ■

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1. Kubelka, J., Hofrichter, J. & Eaton, W. A. *Curr. Opin. Struct. Biol.* **14,** 76–88 (2004).
2. Chen, J., Rempel, D. L. & Gross, M. L. *J. Am. Chem. Soc.* **132,** 15502–15504 (2010).
3. Shaw, D. E. *et al. Science* **330,** 341–346 (2010).
4. Gruebele, M. *C.R. Biol.* **328,** 701–712 (2005).
5. Nölting, B., Golbik, R. & Fersht, A. R. *Proc. Natl Acad. Sci. USA* **92,** 10668–10672 (1995).
6. Ballew, R. M., Sabelko, J. & Gruebele, M. *Proc. Natl Acad. Sci. USA* **93,** 5759–5764 (1996).
7. Gau, B. C., Sharp, J. S., Rempel, D. L. & Gross, M. L. *Anal. Chem.* **81,** 6563–6571 (2009).
8. de Laureto, P. P., De Filippis, V., Di Bello, M., Zambonin, M. & Fontana, A. *Biochemistry* **34,** 12596–12604 (1995).
9. Tsui, V. *et al. Protein Sci.* **8,** 45–49 (1999).

PARASITOLOGY

Nematode debt to bacteria

The transition by certain nematode worms to plant parasitism, and possibly more generally to herbivory, is illuminated by an investigation into how nematodes acquired the protein weapons to penetrate the plant cell wall.

NOAH K. WHITEMAN & ANDREW D. GLOSS

In the *Iliad*[1], Homer chronicles how the Achaeans, after invading Trojan territory, built a mighty wall around their encampment to protect themselves. The Trojans retaliated with a multi-pronged assault, and with heavenly intercession the wall was breached.

According to remarkable findings published in *Proceedings of the National Academy of Sciences* by Danchin *et al.*[2], a similar multi-pronged attack is deployed by nematode worms that parasitize plant roots. Danchin *et al.* studied two lineages of nematode that are obligate plant endoparasites; that is, as an essential part of their life cycle they must breach the plant cell wall and enter living cells. The authors show that, over deep evolutionary time, these nematodes have incorporated into their genomes at least six distinct types of bacterial genes encoding proteins that can modify the plant cell wall. These genes have subsequently undergone extensive gene duplication in the nematode lineages. A clear hypothesis emerging from this study is that the proteins encoded by these genes facilitated the invasion of the plant root, and perhaps herbivory in nematodes more generally.

Nematodes are important ecologically and hyperdiverse evolutionarily; most are parasitic[3]. Among the plant-parasitic nematodes that cause serious crop losses are the two lineages of root nematodes within a group (clade IV) of the Tylenchina, in which endoparasitism in plants evolved independently at least ten times[4]. These lineages — root-knot nematodes (RKN) and cyst nematodes (CN) — each have free-living stages that, as juveniles, invade root tissue, enter individual cells and then migrate within the roots to complete their

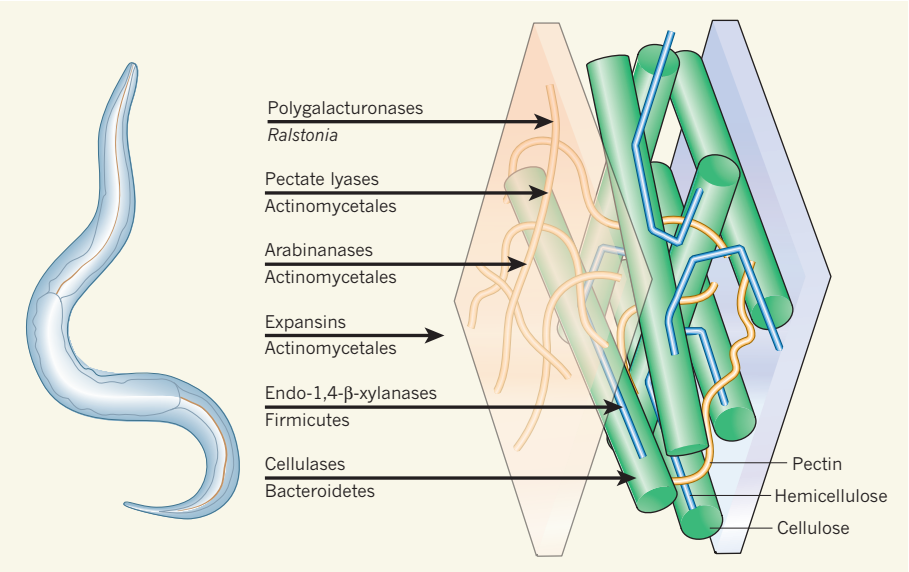

Figure 1 | A multi-pronged assault. Plant-parasitic nematodes possess a diverse suite of proteins capable of degrading plant cell walls. On the basis of phylogenetic analyses of genomic sequence data from nematode lineages, Danchin *et al.*[2] suggest that the distribution of genes encoding six families of cell-wall-modifying proteins is the result of multiple lateral gene transfers from bacteria to ancestral nematodes. The protein families are noted above the arrows, and the likely source bacteria below. The cell-wall targets of attack are pectin (orange), hemicellulose (blue) and cellulose (green).

development. This life history is distinct from that of other plant-parasitic nematodes, which insert structures called stylets into plant cells, but do not invade them.

The primary plant cell wall consists largely of polysaccharides, principally cellulose (a polymer of glucose), hemicellulose and branched polysaccharides including pectins and glycans. Bacteria and fungi are adept at overcoming these barriers and gaining entry to exploit the resources within cells. By contrast, animals are not typically well equipped for breaking down cell walls; to do so, various species — ranging from cows to termites and nematodes — have turned to bacterial genomes for assistance. Some of these arrangements rely on host–symbiont relationships. But a more subtle form of mutualism can occur as a result of lateral gene transfer (LGT), the movement of a gene from one species' genome to another, with subsequent vertical transmission in the new genome[5].

Danchin *et al.*[2] searched whole-genome sequences of RKN and CN for genes encoding proteins that degrade or modify plant cell walls, and subjected them to phylogenetic analysis. The authors identified a diverse array of six families of genes with distinct functional roles in the invasion of plant cell walls (Fig. 1). Although cell-wall-modifying proteins of bacterial origin have been reported previously from plant-parasitic nematodes, the whole-genome data allowed Danchin *et al.* to identify six, probably independent, transfer events and subsequent gene duplications, resulting in the presence of more than 60 loci peppered throughout nematode genomes.

The authors infer that the timing of these events is ancient because the genes have certain features that are not typical of bacteria, indicating a long period of divergence since the original transfer. Their phylogenetic analyses in nearly every case do not support hypotheses other than those indicating a bacterial origin for each gene family within the plant-parasitic nematode lineages.

Remarkably, the closest relatives of the nematode members for all six gene families include genes in soil bacteria, some of which are known plant symbionts or pathogens. This is evidence that potential bacterial donors and nematodes occur together in the same habitat, providing the ecological opportunity for LGT to occur. The sequencing of further plant-parasitic nematode genomes will allow more precise identification of the origin and elaboration of each gene family in relation to the ten or more independent evolutions of plant endoparasitism across the Tylenchina evolutionary tree. There is also the possibility that these genes occur in other, non-endoparasitic, nematode species.

To understand the full implications of these gene transfers, future studies should focus on identifying the functional and ecological role of each protein in model nematode species. Through antibody staining of proteins bearing a laterally acquired cellulose-binding module (CBM2), Danchin *et al.* demonstrate that some of these acquired proteins, in addition to being secreted into surrounding plant tissue, localize to the shells of eggs developing within nematodes. The experimental silencing of laterally acquired cell-wall-modifying proteins has, importantly, been shown to reduce nematode virulence in plants[6]. Whether any of the proteins are also required for successful egg laying remains unclear. Their localization to eggshells suggests that one or more of them may have secondarily evolved roles in egg development, perhaps through an interaction with chitin — a glucose-based polymer found in nematode eggshells that is structurally very similar to cellulose. Interestingly, mechanisms conferring binding specificity for chitin have been identified in a CBM2 domain from an archaeal microorganism[7]. Screening for mutant worms showing abnormal egg development, following the silencing of CBM2-encoding genes, could reveal whether proteins that interact with the cell wall have been co-opted by nematodes to perform novel biochemical roles.

Plant-parasitic nematodes display the most extensive diversifications of functionally related, laterally acquired genes to have been identified in complex eukaryotes (organisms, such as animals, plants and fungi, with a membrane-bound nucleus, in contrast to bacteria and archaea). But ecologically significant LGT events have been discovered in other eukaryotes as well. Convergent strategies to parasitize plants are correlated with LGT from fungi to oomycetes, with a subset of these transferred genes representing secondary transfers from bacteria[8]. Similarly, the acquisition of bacterial genes for anaerobic metabolism has been extensively documented in protozoan parasites[9–11]. Although LGT has been observed in non-parasitic eukaryotes, the cell-wall-modifying proteins in plant-parasitic nematodes represent yet another example of LGT that potentially prompted a major transition to an endoparasitic or herbivorous lifestyle.

The recent explosion of eukaryotic genome sequencing projects will facilitate further discovery of LGT events that could have enabled ecological transitions in environmentally and economically important parasites. Such studies will also offer unique opportunities to determine how laterally acquired genes evolve at both the single-gene and genome-wide scales. ■

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1. Lattimore, R. (transl.) *The Iliad of Homer* (Univ. Chicago Press, 1951).
2. Danchin, E. G. J. *et al. Proc. Natl Acad. Sci. USA* **107,** 17651–17656 (2010).
3. Hugot, J.-P., Baujard, P. & Morand, S. *Nematology* **3,** 199–208 (2001).
4. Holterman, H. *et al. Phytopathology* **99,** 227–235 (2009).
5. Keeling, P. J. & Palmer, J. D. *Nature Rev. Genet.* **9,** 605–618 (2008).
6. Lilley, C. J., Bakhetia, M., Charlton, W. L. & Urwin, P. E. *Mol. Plant Pathol.* **8,** 701–711 (2007).
7. Nakamura, T. *et al. J. Mol. Biol.* **381,** 670–680 (2008).
8. Richards, T. A. *et al. Curr. Biol.* **16,** 1857–1864 (2006).
9. Loftus, B. *et al. Nature* **433,** 865–868 (2005).
10. Carlton, J. M. *et al. Science* **315,** 207–212 (2007).
11. Morrison, H. G. *et al. Science* **317,** 1921–1926 (2007).