Plant degradation

A nematode expansin acting on plants

Expansin proteins, which have so far been identified only in plants, rapidly induce extension of plant cell walls by weakening the non-covalent interactions that help to maintain their integrity[1]. Here we show that an animal, the plant-parasitic roundworm *Globodera rostochiensis*, can also produce a functional expansin, which it uses to loosen cell walls when invading its host plant. As this nematode is known to be able to disrupt covalent bonds in plant cell walls[2,3], its accompanying ability to loosen non-covalent bonds challenges the prevailing view that animals are genetically poorly equipped to degrade plant cell walls.

The plant cell wall is a rigid network of interwoven polymers, and many organisms that use plants as a food source use a variety of glycanase enzymes to break covalent bonds in this polysaccharide-based structure. We used complementary DNA–AFLP (for amplified fragment-length polymorphism)-based transcript profiling of synchronized life stages as a starting point to identify the proteins that are used by *G. rostochiensis* to degrade cell walls. A cDNA fragment, KT21 (137 nucleotides), was found to be predominantly expressed in infective second-stage juveniles (J2) and the corresponding full-length cDNA (*Gr-Exp1*; accession number AJ311901; length, 1,061 base pairs) encoded a protein of 271 amino acids that has a predicted amino-terminal signal peptide for secretion.

Similarity searches (BLASTP) indicated that two distinct regions are present in the predicted mature protein. Domain 1 (residues 26–118) shows significant similarity to the carbohydrate-binding module family II of endoglucanases (AF056110, BAB68522 and AF323087; 39–43% identity and expectation values (*E*-value) from 2.0×10^{-12} to 0.0008) from various nematode species. Domain 2 (residues 150–271) showed significant `similarity to a β-expansin-like protein (*PPAL*) from *Nicotiana tabacum* (AAG52887; *E*-value, 2.2×10^{-5}) and a putative β-expansin from *Arabidopsis thaliana* (O04484; *E*-value, 6×10^{-4}). A local alignment of domain 2 with these β-expansins indicated the presence of a series of conserved cysteine residues, the HFD motif (although Gr-EXP1 harbours a conservative substitution (F→V)) and other conserved motifs[4].

Whole-mount *in situ* hybridization was carried out on pre-parasitic infective second-stage juveniles of *G. rostochiensis*[5]. Antisense cDNA probes amplified from the *Gr-Exp1* cDNA (nucleotides 54–427) hybridized specifically to the subventral oesophageal glands (Fig. 1a). Gr-EXP1 antiserum reacted strongly with nematode secretions, induced by potato-root diffusate, on dot blots (results not shown). We conclude that Gr-EXP1 is produced in the subventral oesophageal glands of infective juveniles, and that Gr-EXP1 and cell-wall-degrading enzymes[6] are secreted simultaneously.

Cell-wall-extension activity[7] was demonstrated in homogenates of infective second-stage juveniles, and was much stronger on wheat than on cucumber (Fig. 1b). Homogenates of adult females showed no such activity (Fig. 1b). Protein extracts from mature leaves of *Gr-Exp1*-transformed tobacco produced significantly more expansin activity on wheat coleoptiles than did empty-vector controls (Fig. 1c). On the basis of the significant similarity of Gr-EXP1 to putative β-expansins from *N. tabacum* and *A. thaliana*, the presence of several amino-acid motifs that are characteristic of expansins, and the potent expansin activity of both recombinant Gr-EXP1 and nematode homogenates on plant-cell walls, we conclude that *Gr-Exp1* encodes a functional expansin.

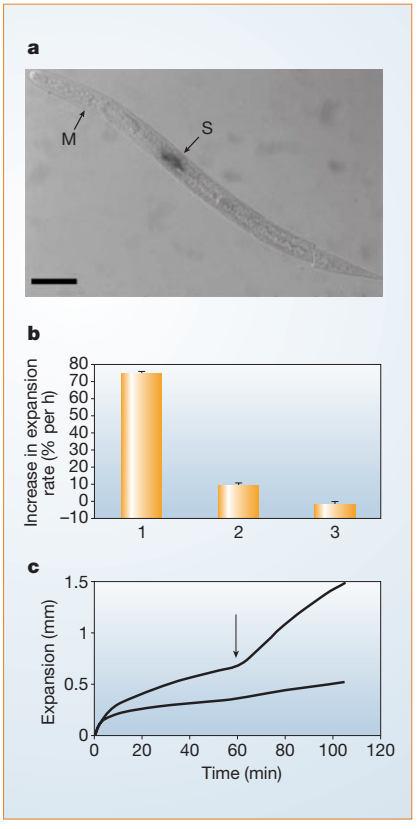

Figure 1 Localization of the nematode *Gr-Exp1* transcript and extension activity of Gr-EXP1 on plant cell walls. **a,** *In situ* hybridization labelling pattern in infective second-stage juveniles (J2) using a *Gr-Exp1* antisense probe. Arrows indicate the subventral glands (S) and the metacorpus (M), respectively. Scale bar, 20 μm. **b,** Effects of nematode homogenate on the extension rate of heat-inactivated wheat coleoptiles and cucumber hypocotyls. Bar 1, J2 homogenate on wheat coleoptiles; bar 2, J2 homogenate on cucumber hypocotyls; bar 3, young female homogenate on wheat coleoptiles. **c,** Extension curves for a wheat coleoptile treated with mature-leaf extracts from *Gr-Exp1*-harbouring (upper curve) and empty-vector-harbouring (lower curve) tobacco plants. Arrow indicates the point at which the control buffer was replaced by leaf extract.

Sequences that remotely resemble expansins have been found in various taxa outside the plant kingdom[8–10], but cell-wall-loosening activity has not been demonstrated for any of the corresponding proteins. To our knowledge, *Gr-Exp1* is the first non-plant gene found to have the structural and functional characteristics that define the expansin superfamily.

This finding undermines the previously accepted view that animals are poorly equipped for degrading plant cell walls. When cell-wall-degrading enzymes and expansin are simultaneously secreted by the cyst nematode, the activity of expansin may increase the accessibility of cell-wall components to glycanases. This might account for the remarkably high rate (about 2 min per cell layer) at which cyst nematodes can penetrate the host plant.

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1. Cosgrove, D. J. *Nature* **407,** 321–326 (2000).
2. Popeijus, H. *et al. Nature* **406,** 36–37 (2000).
3. Jaubert, S., Laffaire, J. B., Abad, P. & Rosso, M. N. *FEBS Lett.* **522,** 109–112 (2002).
4. Cosgrove, D. J. *Curr. Opin. Plant Biol.* **3,** 73–78 (2000).
5. Qin, L. *et al. Mol. Plant Microbe Interact.* **13,** 830–836 (2000).
6. Davis, E. L. *et al. Annu. Rev. Phytopathol.* **38,** 365–396 (2000).
7. McQueen, M. S., Durachko, D. M. & Cosgrove, D. J. *Plant Cell* **4,** 1425–1433 (1992).
8. Laine, M. J. *et al. Physiol. Mol. Plant Pathol.* **57,** 221–233 (2000).
9. Saloheimo, M. *et al. Eur. J Biochem.* **269,** 4202–4211 (2002).
10. Xu, B., Hellman, U., Ersson, B. & Janson, J. C. *Eur. J Biochem* **267,** 4970–4977 (2000).

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