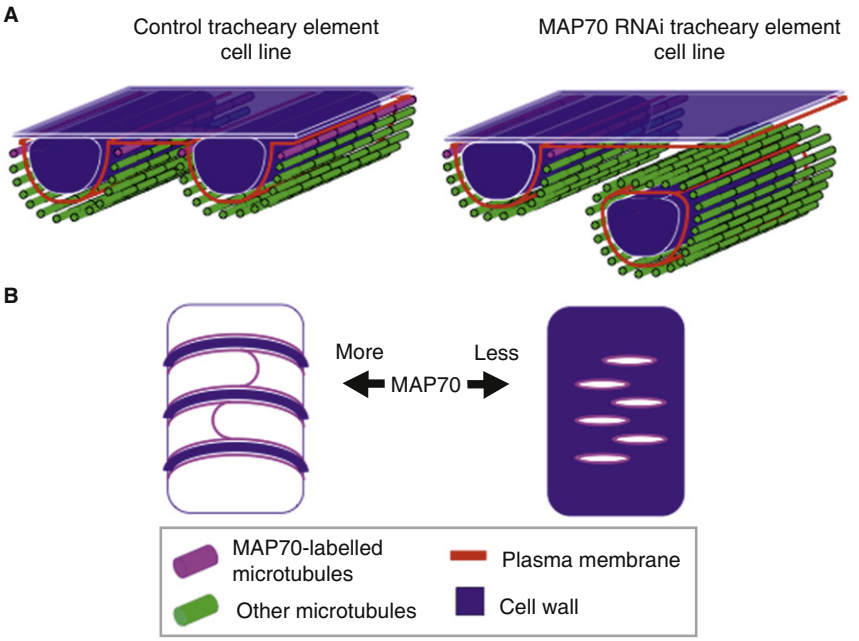

Figure 2. A schematic model of how MAP70 proteins might position cortical microtubules and control cell wall patterning in developing tracheary elements.

(A) In control tracheary elements, microtubules (green) form a U-shaped mould while AtMAP70-5 and -1 (light purple) decorate those microtubules flanking the thickenings adjacent to the primary cell wall (purple). RNAi silencing causes the separation of the thickening and its associated microtubules from the primary cell wall. (B) Schematic representation of how the secondary thickening pattern might be regulated by modulating the amount of AtMAP70-5 and -1. Larger amounts of AtMAP70 increase the spacing between thickenings, resulting in an increased percentage of spiral patterns among the tracheary elements. Reduction of the amount of AtMAP70 reduces the spacing between thickenings, leaving only unthickened pits within the secondary wall.

results indicate that the abundance of the AtMAP70-1–AtMAP70-5 complex in the cell is sufficient to explain the different cell wall patterns. It remains unclear, however, whether the relative abundance of the AtMAP70s also explains the cell wall patterns in wild-type proto- and metaxylem cells and whether the transcription factors VND7 and VND6 differentially regulate their expression levels. Another, rather bizarre finding was that, in the silenced lines for either AtMAP70, about half of the thickenings were detached from the surface and formed internalized strands consisting of cell wall material surrounded by plasma membrane and microtubules. The authors propose that AtMAP70-decorated microtubules may define the boundary between the thickenings and the primary cell wall. In the absence of such a boundary in the RNAi lines, the thickening with its associated plasma membrane and microtubules can disconnect from the rest of the plasma membrane (Figure 2A). Live cell imaging during tracheary element differentiation in the silenced lines should provide insights into the mechanism of the internalization of the thickenings.

In conclusion, this study provides new insights into how cell wall deposition patterns during wood formation can be controlled by microtubule-binding proteins.

References

1. Fukuda, H., and Komamine, A. (1980). Establishment of an experimental system for the study of tracheary element differentiation from single cells isolated from the mesophyll of *Zinnia elegans*. Plant Physiol. *65*, 57–60.
2. Kubo, M., Udagawa, M., Nishikubo, N., Horiguchi, G., Yamaguchi, M., Ito, J., Mimura, T., Fukuda, H., and Demura, T. (2005). Transcription switches for protoxylem and metaxylem vessel formation. Genes Dev. *19*, 1855–1860.
3. Demura, T., and Fukuda, H. (2007). Transcriptional regulation in wood formation. Trends Plant Sci. *12*, 64–70.
4. Crowell, E.F., Bischoff, V., Desprez, T., Rolland, A., Stierhof, Y.D., Schumacher, K., Gonneau, M., Hofte, H., and Vernhettes, S. (2009). Pausing of Golgi bodies on microtubules regulates secretion of cellulose synthase complexes in *Arabidopsis*. Plant Cell *21*, 1141–1154.
5. Gutierrez, R., Lindeboom, J.J., Paredez, A.R., Emons, A.M., and Ehrhardt, D.W. (2009). *Arabidopsis* cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartments. Nat. Cell Biol. *11*, 797–806.
6. Paredez, A.R., Persson, S., Ehrhardt, D.W., and Somerville, C.R. (2008). Genetic evidence that cellulose synthase activity influences microtubule cortical array organization. Plant Physiol. *147*, 1723–1734.
7. Oda, Y., Mimura, T., and Hasezawa, S. (2005). Regulation of secondary cell wall development by cortical microtubules during tracheary element differentiation in *Arabidopsis* cell suspensions. Plant Physiol. *137*, 1027–1036.
8. Pesquet, E., Korolev, A.V., Calder, G., and Lloyd, C.W. (2010). The microtubule-associated protein AtMAP70-5 regulates secondary wall patterning in *Arabidopsis* wood cells. Curr. Biol. *20*, 744–749.

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Cellular Homeostasis: Coping with ER Overload During an Immune Response

Host cells secrete antimicrobial proteins *en masse* to counter extracellular pathogens, placing a strain on the endoplasmic reticulum. The interplay between defence and cellular homeostasis has now been dissected genetically in *Caenorhabditis elegans*.

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Immunologists are an isolated breed. Their work is shrouded in mystery, with impenetrable codes and such a specialized vocabulary that it takes the uninitiated years to be fully versant in the discipline. In part, this is a consequence of a long-standing

methodology in immunological research: extracting circulating cells from an organism and studying them *in vitro*. Increasingly, this is changing. One does still need to learn, for example, that CD29 is an integrin, CD56 is the adhesion molecule NCAM, and CD220 is the insulin receptor. But the application of intravital microscopy and the growing use of direct genetic screens for immunological traits are moving the study of mammalian immunology back into a real physiological context. Studying innate immunity in whole animals allows for its integration with the ensemble of cellular and organismal physiology (for recent examples, using the nematode *Caenorhabditis elegans*, see [1–3]). This integration is well illustrated by recent work from Dennis Kim's lab [4] that now shows that the transcriptional response to infection in *C. elegans* is tied to the molecular machinery that governs homeostasis in the endoplasmic reticulum (ER).

The maturation and folding of proteins is a complicated affair. It is estimated that one-third of all newly synthesized proteins fail to mature and get recycled. The molecular machinery that ensures this quality control runs at close to its full capacity [5]. At times of increased protein synthesis and secretion or as a result of defects in ER function, the system can become over-stretched. This may occur, for example, during development, or during an immune response. In such situations, a series of back-up measures — collectively known as the unfolded protein response (UPR) — then comes into play. These vital mechanisms ensure cell survival and are largely conserved from yeast to mammals [6].

In broad terms, the UPR in higher organisms is made up of three components. The first involves slowing down translation globally, so that the entry of neo-synthesized proteins into the ER is reduced, limiting the strain on the system. The second results in the specific upregulation of the expression of chaperones, such as members of the heat-shock protein family, which aid protein folding in the ER. The third is an increase in the degradative capacity of the cell, to eliminate misfolded proteins. These mechanisms act sequentially, and maximize the chances that the cell will overcome a transient overload of the ER [6].

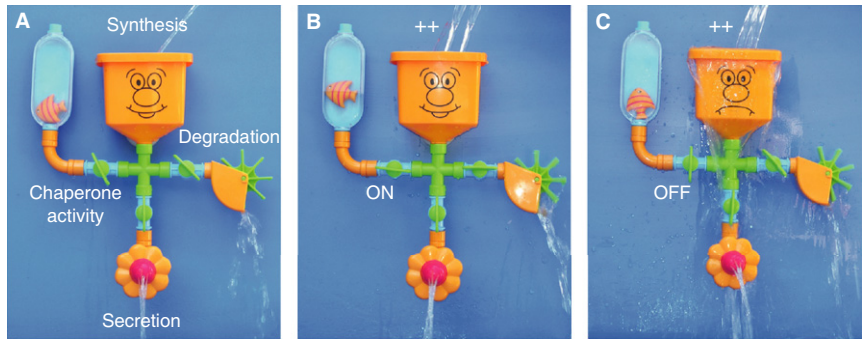

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Figure 1. A bath-toy analogy for the role of the UPR in coping with high levels of protein synthesis.

(A) Under normal conditions, in adults, the rate of protein synthesis (water from above) does not place undue strain on the ER (orange reservoir). Most proteins are successfully secreted (downward flow from the flower nozzle), while some are degraded (flow via the water-wheel). (B) During development, or under pathological situations, such as infection, the rate of protein synthesis increases (increased flow of water). To counter-balance this, before the general synthesis rate is diminished by a feedback mechanism (not shown), as part of the UPR protein degradation increases (represented by opening of the tap on the right) and chaperone activity increases (represented by opening of the tap on the left), providing adequate buffering (shown by the filling of the secondary reservoir on the left) to allow for increased protein secretion. (C) If the UPR does not function properly, for example if XBP-1 activity is blocked (tap on the left stays closed) and protein degradation does not increase (tap on the right stays at its initial position), then the ER is no longer able to cope with the increased protein synthesis (orange reservoir overflows). If protein synthesis does not increase drastically during an infection, for example in *C. elegans* when the p38 MAP kinase pathway is blocked, then even if the UPR is not fully functional the system is not overloaded, as in (A).

On the basis of genetic studies and expression profiles of different mutants, the UPR in *C. elegans* has been divided into constitutive and inducible pathways. The constitutive branch functions during development. Its importance has been demonstrated by the fact that development is arrested at an early stage for UPR-deficient mutants. These mutants that lack the capacity to increase expression of chaperones or of the enzymes required to degrade misfolded proteins then die, apparently because of necrotic cell death. It has been speculated that stress on the ER leads to release of calcium stores and that this provides the trigger for the subsequent necrosis [7,8].

The changes in gene expression that accompany the UPR involve a very particular process, common to *C. elegans* and vertebrates. Activation of the endoribonuclease IRE-1 leads to the production of an alternatively spliced isoform of the mRNA of the transcription factor XBP-1 [9]. Exposure to drugs such as tunicamycin, which interferes with the glycosylation of proteins in the ER, triggers the production of the UPR-specific form of XBP-1, thereby leading, in *C. elegans,* to chaperone gene expression throughout the organism [7–9]. It has been shown that feeding the bacterial pore-forming toxin Cry5B to *C. elegans* similarly upregulates chaperone expression, but only in the intestine. Interestingly, and again in contrast with the effect of tunicamycin, in the presence of Cry5B, the activation of IRE-1 requires a conserved p38 MAP kinase signalling cascade [1]. This is a central part of *C. elegans* defences both in the intestine [10–12] and in the epidermis [13,14]. These results have led to the suggestion that the UPR could be an integral part of the nematode's innate immune system [1].

Such a hypothesis has now been refined in the recent work by Kim and colleagues [4], who found that the well-characterized Gram-negative pathogen *Pseudomonas aeruginosa* also induces IRE-1-mediated splicing of *xbp-1* mRNA and consequent chaperone gene expression in a p38 MAP kinase-dependent manner. These authors were able to trace the regulatory pathway one step further, by identifying a conserved transcription

factor called ATF-7 as a target of p38 MAP kinase signalling. They showed that ATF-7 is required for the induction of chaperone gene expression seen upon *P. aeruginosa* infection. They also demonstrated that the IRE-1–XBP-1 branch of the UPR is required during development when *P. aeruginosa* is present. The fact that pathogenic bacteria caused the developmental arrest of *xbp-1* mutants, even when mixed at a low concentration with the normal food source, *Escherichia coli*, suggests that it is a consequence of the infection by *P. aeruginosa* and not of a nutritional defect [4]. *P. aeruginosa* does, however, provoke evident changes in intestinal morphology and could impair digestive functions. It remains to be formally demonstrated that changes in intestinal cell physiology do not contribute to the observed developmental arrest.

It is not clear exactly why the *xbp-1* mutants are especially susceptible to *P. aeruginosa*. The bacteria accumulated in the intestines of the mutants at a rate that was comparable to that observed in wild-type animals. An analysis of intestinal cell ultrastructure by electron microscopy, however, revealed that in contrast to wild-type larvae, the ER in mutant larvae had an abnormal morphology [4] that was similar to that seen in worms treated with tunicamycin, i.e. suffering from an excess of misfolded proteins. As mentioned above, during development, loss of an efficient UPR is sufficient to cause developmental arrest. So the combination of infection and incapacity to mount a normal UPR is sufficient to lead to growth arrest and death. Triggering of the UPR in wild-type animals leads to necrotic cell death. Interestingly, although blocking necrosis does increase resistance of *C. elegans* to some bacterial pathogens [15], it did not alter the precocious death of *xbp-1* mutants cultured on *P. aeruginosa* [4].

Further insight into the phenomenon was gained by the analysis of double mutants deficient for both the UPR and p38 MAP kinase signalling. Although it was not reported whether the accumulation of *P. aeruginosa* was altered in these double mutants, these animals developed and survived much better in the presence of the pathogen than the UPR single mutant; indeed, their survival was comparable to that of the p38 MAP kinase single mutant [4]. Previous studies in *C. elegans* have shown that the activity of p38 MAP kinase is negatively regulated by a specific phosphatase [11]. Remarkably, even under standard culture conditions, knocking down the activity of this phosphatase was sufficient to provoke a UPR, as witnessed by an increase in chaperone gene expression. In other words, activating the p38 MAP kinase appears sufficient to induce ER overload. Additionally, combining loss of function of the phosphatase with mutation of *xbp-1* caused a developmental arrest under normal culture conditions, like that seen for *xbp-1* mutants exposed to *P. aeruginosa*. The phenotypes of the *xbp-1*–p38 MAP kinase double mutant were suppressed when phosphatase activity was abrogated [4]. Exposure to *P. aeruginosa* causes the rapid induction of a very large number of genes, many of which are predicted to encode secreted proteins [4,16]. The most parsimonious explanation of these results is therefore that infection by *P. aeruginosa* provokes a greatly increased flux of proteins through the ER, dependent upon a p38 MAP kinase cascade. In normal worms, this leads to a UPR, which guarantees cell survival. If the UPR is compromised, *C. elegans* can no longer cope with the increased demand placed on its ER, and so is killed by its own defences (Figure 1).

It will clearly be interesting to establish how general this phenomenon is, both in *C. elegans*, where several pathways cooperate to mediate resistance to *P. aeruginosa* [17] and different types of pathogens are known to colonize or infect the intestine (e.g. [18,19]), as well as in other species, including mammals. In vertebrates, the situation is likely to be far more complex as altering ER function also affects antigen presentation, cytokine production, as well as the survival of cell types involved in immunity [20]. Nevertheless, the study by Kim and colleagues [4] is an elegant example of the interest in studying the host response to infection in a whole-animal model, and suggests that researchers will need to continue their efforts to integrate established discrete models of immune system function into a more holistic physiological view.

References

1. Bischof, L.J., Kao, C.Y., Los, F.C., Gonzalez, M.R., Shen, Z., Briggs, S.P., van der Goot, F.G., and Aroian, R.V. (2008). Activation of the unfolded protein response is required for defenses against bacterial pore-forming toxin in vivo. PLoS Pathog. *4*, e1000176.
2. Rohlfing, A.K., Miteva, Y., Hannenhalli, S., and Lamitina, T. (2010). Genetic and physiological activation of osmosensitive gene expression mimics transcriptional signatures of pathogen infection in *C. elegans*. PLoS ONE *5*, e9010.
3. Lee, K.Z., Kniazeva, M., Han, M., Pujol, N., and Ewbank, J.J. (2010). The fatty acid synthase *fasn-1* acts upstream of WNK and Ste20/GCK-VI kinases to modulate antimicrobial peptide expression in *C. elegans* epidermis. Virulence, in press.
4. Richardson, C.E., Kooistra, T., and Kim, D.H. (2010). An essential role for XBP-1 in host protection against immune activation in *C. elegans*. Nature *463*, 1092–1095.
5. Yewdell, J.W. (2001). Not such a dismal science: the economics of protein synthesis, folding, degradation and antigen processing. Trends Cell Biol. *11*, 294–297.
6. Mori, K. (2009). Signalling pathways in the unfolded protein response: development from yeast to mammals. J. Biochem. *146*, 743–750.
7. Shen, X., Ellis, R.E., Lee, K., Liu, C.Y., Yang, K., Solomon, A., Yoshida, H., Morimoto, R., Kurnit, D.M., Mori, K., et al. (2001). Complementary signaling pathways regulate the unfolded protein response and are required for *C. elegans* development. Cell *107*, 893–903.
8. Shen, X., Ellis, R.E., Sakaki, K., and Kaufman, R.J. (2005). Genetic interactions due to constitutive and inducible gene regulation mediated by the unfolded protein response in *C. elegans*. PLoS Genet. *1*, e37.
9. Calfon, M., Zeng, H., Urano, F., Till, J.H., Hubbard, S.R., Harding, H.P., Clark, S.G., and Ron, D. (2002). IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. Nature *415*, 92–96.
10. Kim, D.H., Feinbaum, R., Alloing, G., Emerson, F.E., Garsin, D.A., Inoue, H., Tanaka-Hino, M., Hisamoto, N., Matsumoto, K., Tan, M.W., et al. (2002). A conserved p38 MAP kinase pathway in *Caenorhabditis elegans* innate immunity. Science *297*, 623–626.
11. Kim, D.H., Liberati, N.T., Mizuno, T., Inoue, H., Hisamoto, N., Matsumoto, K., and Ausubel, F.M. (2004). Integration of *Caenorhabditis elegans* MAPK pathways mediating immunity and stress resistance by MEK-1 MAPK kinase and VHP-1 MAPK phosphatase. Proc. Natl. Acad. Sci. USA *101*, 10990–10994.
12. Ren, M., Feng, H., Fu, Y., Land, M., and Rubin, C.S. (2009). Protein kinase D (DKF-2), a diacylglycerol effector, is an essential regulator of *C. elegans* innate immunity. Immunity *30*, 521–532.
13. Pujol, N., Zugasti, O., Wong, D., Couillault, C., Kurz, C.L., Schulenburg, H., and Ewbank, J.J. (2008). Anti-fungal innate immunity in *C. elegans* is enhanced by evolutionary diversification of antimicrobial peptides. PLoS Pathog. *4*, e1000105.
14. Ziegler, K., Kurz, C.L., Cypowyj, S., Couillault, C., Pophillat, M., Pujol, N., and Ewbank, J.J. (2009). Antifungal innate immunity in *C. elegans*: PKCdelta links G protein signaling and a conserved p38 MAPK cascade. Cell Host Microbe *5*, 341–352.
15. Wong, D., Bazopoulou, D., Pujol, N., Tavernarakis, N., and Ewbank, J.J. (2007). Genome-wide investigation reveals pathogen-specific and shared signatures in the response of *Caenorhabditis elegans* to infection. Genome Biol. *8*, R194.
16. Shapira, M., Hamlin, B.J., Rong, J., Chen, K., Ronen, M., and Tan, M.W. (2006). A conserved role for a GATA transcription factor in regulating epithelial innate immune responses. Proc. Natl. Acad. Sci. USA *103*, 14086–14091.

17. Estes, K.A., Dunbar, T.L., Powell, J.R., Ausubel, F.M., and Troemel, E.R. (2010). bZIP transcription factor *zip-2* mediates an early response to *Pseudomonas aeruginosa* infection in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA *107*, 2153–2158.
18. Hodgkin, J., Kuwabara, P.E., and Corneliussen, B. (2000). A novel bacterial pathogen, *Microbacterium nematophilum*, induces morphological change in the nematode *C. elegans*. Curr. Biol. *10*, 1615–1618.
19. Troemel, E.R., Felix, M.A., Whiteman, N.K., Barriere, A., and Ausubel, F.M. (2008). Microsporidia are natural intracellular parasites of the nematode *Caenorhabditis elegans*. PLoS Biol. *6*, 2736–2752.
20. Pierre, P. (2009). Immunity and the regulation of protein synthesis: surprising connections. Curr. Opin. Immunol. *21*, 70–77.

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