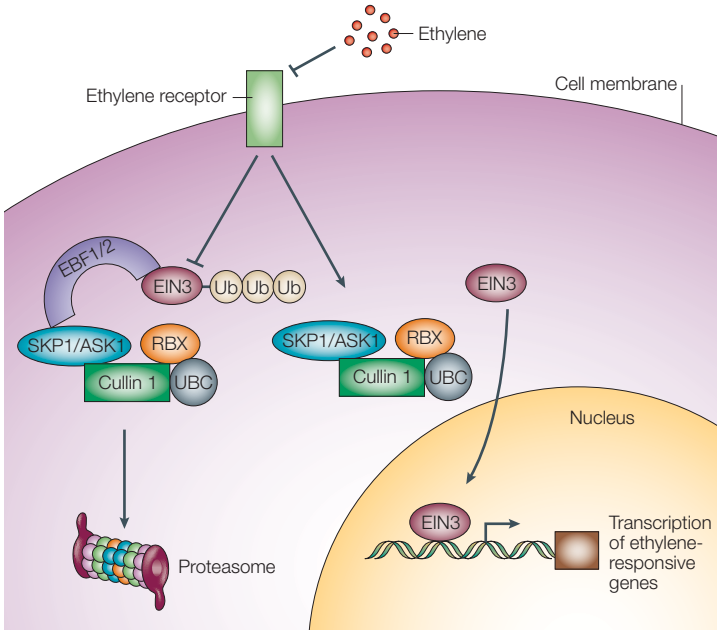
PLANT CELL BIOLOGY

Protein degradation protects plants

Plants can't move to a new environment if the one they're in becomes inhospitable, so they have evolved mechanisms to rapidly respond to external stimuli. The gaseous plant hormone ethylene triggers various protective responses in *Arabidopsis thaliana* by activating a signalling cascade that coordinates the transcription of ethylene-responsive genes, which are regulated by the transcription factor EIN3. In *Cell*, two studies — by Guo and Ecker, and by Genschik and colleagues — fill some gaps in this signalling pathway and show that ethylene prevents constitutive EIN3 degradation by the ubiquitin/proteasome pathway.

Normally, EIN3 is barely detectable in *Arabidopsis*, but Guo and Ecker found that EIN3 levels increased rapidly in response to the hydrocarbon ethylene. *EIN3* expression is not regulated at the level of transcription and, as blocking protein synthesis with cyclohexamide had no effect on the ethylene response, *de novo* protein synthesis is also not responsible for the increase in EIN3. When ethylene-treated seedlings were placed in hydrocarbon-free air, EIN3 levels decreased markedly within 30 minutes, which indicates that EIN3 is short-lived. The ubiquitin/proteasome pathway is involved in rapid protein degradation, and EIN3 levels increased quickly in *Arabidopsis* cultures that were treated with the proteasome-specific inhibitors MG132 and MG115. This indicates that EIN3 degradation is proteasome dependent. When EIN3 was tagged with green fluorescent protein (GFP), both ethylene and MG132 treatment of transgenic *Arabidopsis* plants were seen to increase the accumulation of EIN3–GFP in the nucleus. Taken together, these results show that EIN3 is constitutively expressed and continuously degraded by a proteasome-dependent mechanism, and that ethylene signalling blocks this degradation and allows EIN3 to accumulate.



The ubiquitin/proteasome pathway of protein degradation is under the control of the ubiquitin-ligating SCF (SKP1/Cullin/F-box protein) complex. The F-box proteins confer substrate specificity to SCF and function as key regulators of plant-hormone signalling pathways. Guo and Ecker used genome-wide microarray experiments to identify F-box proteins that are regulated by ethylene, and two of these bound to EIN3 in yeast two-hybrid assays — EIN3-binding F-box protein 1 and 2 (EBF1 and EBF2). In a separate study, Genschik and colleagues identified the same two EIN3-binding proteins, also using yeast two-hybrid assays, and they confirmed the interaction with EIN3 using *in vitro* pull-down assays. In addition, both groups showed that ASK1, another subunit of the *Arabidopsis* SCF complex, associates with EBF1 and EBF2, which indicates that the proteins are part of the SCF complex and that they might target EIN3 for degradation by the ubiquitin/proteasome pathway.

Both groups used similar approaches to determine the role of EBF1 and EBF2 in the ethylene response. *ebf1* and *ebf2* mutant seedlings and *ebf1 ebf2* double-mutant seedlings show accumulation of EIN3 and the classic 'triple response' to ethylene — short hypocotyls, short roots and exaggerated apical hooks — so, both proteins negatively regulate the ethylene response. This function is dependent on EIN3, as *ebf1 ebf2 ein3* triple mutants show no response to ethylene. By contrast, overexpression of EBF1 and EBF2 causes ethylene insensitivity and impaired accumulation of EIN3, confirming that EBF1 and EBF2 are negative regulators of the ethylene pathway.

Negative regulation and regulated protein degradation are emerging as common themes in plant signalling. These two studies strengthen the role of negative regulation in hormone signalling and provide a missing link between the ethylene and the ubiquitin/proteasome pathway.

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References and links

ORIGINAL RESEARCH PAPERS Guo, H. & Ecker, J. R. Plant responses to ethylene gas are mediated by $SCF^{EBF1/EBF2}$-dependent proteolysis of EIN3 transcription factor. *Cell* **115**, 667–677 (2003) | Potuschak, T. *et al*. EIN3-dependent regulation of plant ethylene hormone signaling by two *Arabidopsis* F box proteins: EBF1 and EBF2. *Cell* **115**, 679–689 (2003)

WEB SITES

Joseph Ecker's laboratory: http://qtlpc.salk.edu/pbio/Web/ecker.html

Pascal Genschik's laboratory: http://ibmp.u-strasbg.fr/dep_div_cell/genschik.html

IN THE NEWS

Worming out breast cancer

Scientists are one step closer to understanding the biology of breast cancer after discovering orthologues of the tumour suppressor BRCA1 and its binding partner BARD1 in the nematode *Caenorhabditis elegans*. These two proteins are involved in repairing damaged DNA, thereby preventing cancer from occurring. But, almost a decade after their discovery, how they carry out this function remains a mystery.

Simple organisms, such as *C. elegans*, are useful for analysing molecular pathways, but previous studies in yeast, flies and *C. elegans* had failed to uncover BRCA1 orthologues. A team from Cancer Research UK, together with American and German researchers, have now identified the *C. elegans* orthologue of BARD1, BRD-1, and used this in yeast two-hybrid assays to identify its binding partner BRC-1. "Previously, scientists have looked through the genome of the nematode worm but found no equivalent to the human BRCA1, so our new finding is somewhat unexpected but very exciting" said Simon Boulton from Cancer Research UK (The Press Association, 5 January 2004).

The research, published in *Current Biology* (6 January 2004), shows that nematodes that lack BRC-1 or BRD-1 have a similar phenotype to human cells that lack BRCA1, and exposing these nematodes to γ-irradiation indicated a role for the proteins in DNA repair. Robert Souhami, Director of Clinical and External Affairs at Cancer Research UK, hopes that "Studying the BRCA1 counterpart in the worm will accelerate our understanding of how defects in this gene can lead to breast cancer and in the future will offer possibilities for prevention and treatment." (BBC News, 6 January 2004).

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