TAPPING INTO THE MECHANICAL LINK OF EXPANSINS IN LEAF MORPHOGENESIS

Hoe -Han Goh†, Nic Mullin‡, Alexis Peaucelle¤, Andreas Backhaus†, Jamie Hobbs‡ and Andrew Fleming†

†Dept. Animal and Plant Sciences, University Sheffield, UK, ‡Dept. Physics and Astronomy, University of Sheffield, UK, ¤INRA Versailles, Versailles, France

Introduction

Plant development relies largely on cell expansion. Cell wall loosening is a pre-requisite for cell expansion in which expansins, a conserved family of non-enzymatic proteins, play a major role[1,2]. Ectopic expression of expansin at meristem and leaf flank has been used to induce leaf initiation and aberrant leaf shape[3,4]. However, to what extent this reflects an *in vivo* function for expansins remains unclear. Indeed, the role of cell wall mechanics in controlling morphogenesis remains debatable.

Aims:

- To determine the role of expansins in leaf morphogenesis
- To determine the role of plant cell wall in controlling leaf growth and form

Project outline:

1. Functional analysis of expansins involve in leaf morphogenesis
2. *In vivo* quantification of expansin activity
3. Utilisation of a leaf growth model based on viscoelastic nature of plant cell wall in testing various hypotheses regarding mechanical force and morphogenesis

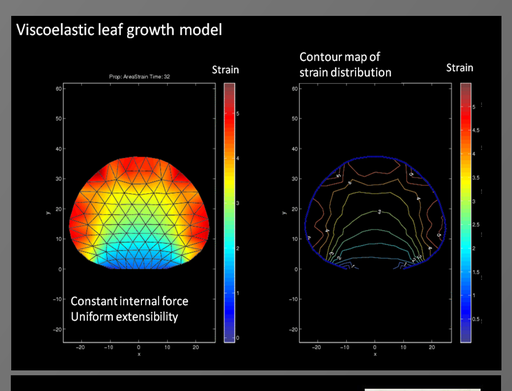
Hypotheses

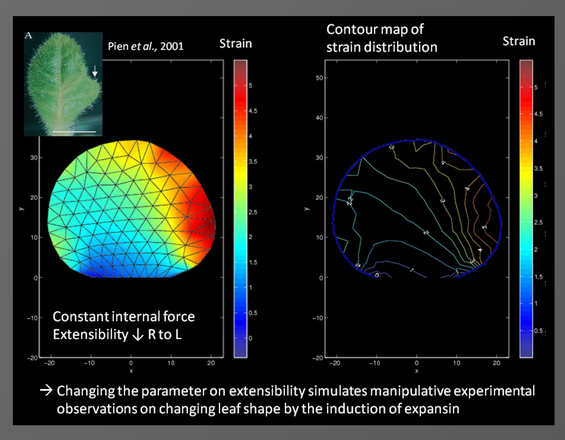
1. The cell wall protein expansins function in differential growth during leaf development
2. The differential regulation of expansin expression leads to organ-level coordination of leaf size and shape

→ alterations of plant cell wall properties modulate leaf growth

Model

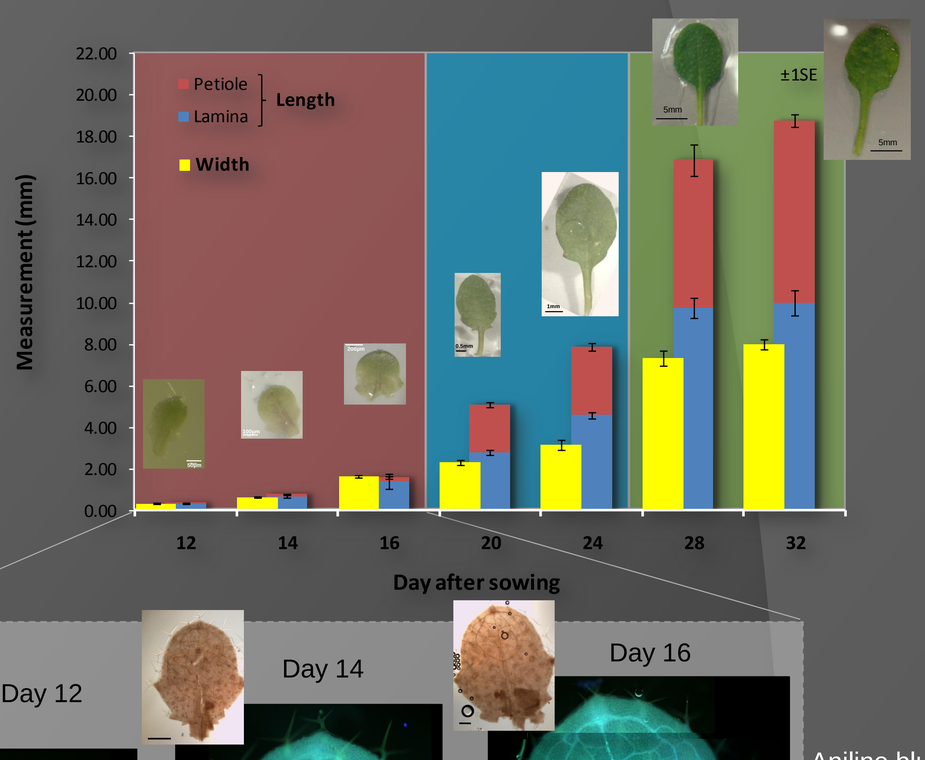
Plant tissue can be modelled as growing network of viscoelastic springs (cell walls) with strain force generated by internal turgor pressure to explore general cellular behaviour during leaf development.

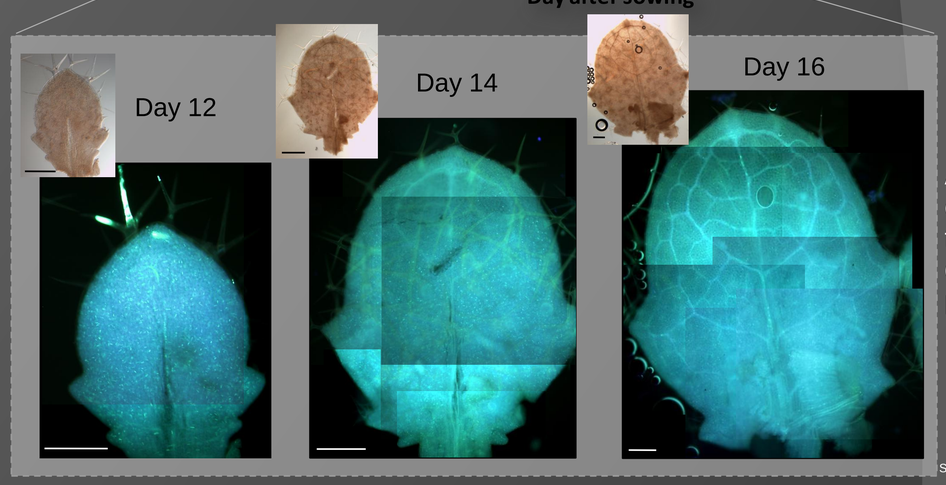




Leaf staging for expansin expression study

Measurements on leaf number 6 of *Arabidopsis thaliana* Col-0 show that leaf development can be categorised into three phases: cell division predominant stage, cell expansion stage and mature stage.



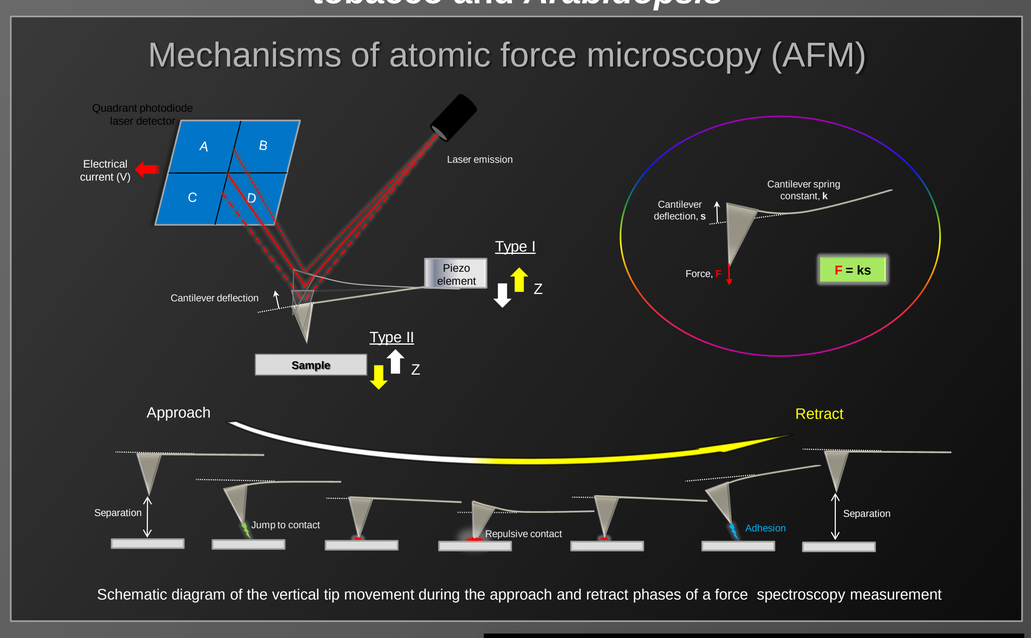


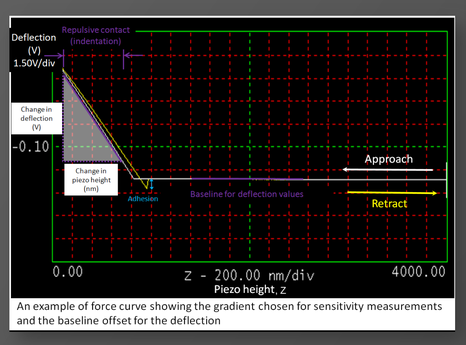
Aniline blue staining of callose which formed during cell division indicating planes of cell division

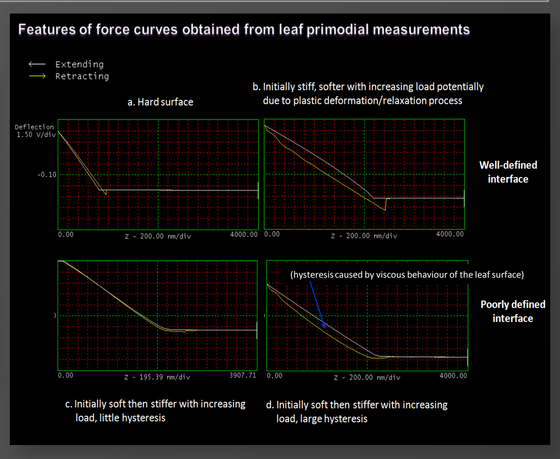
Scale bars = 100µm

The use of AFM for *in vivo* quantification of plant cell wall extensibility

Atomic force microscopy can be used to characterise the mechanical properties of cell wall during early leaf growth in tobacco and *Arabidopsis*

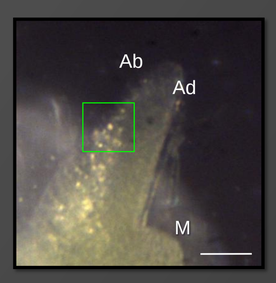


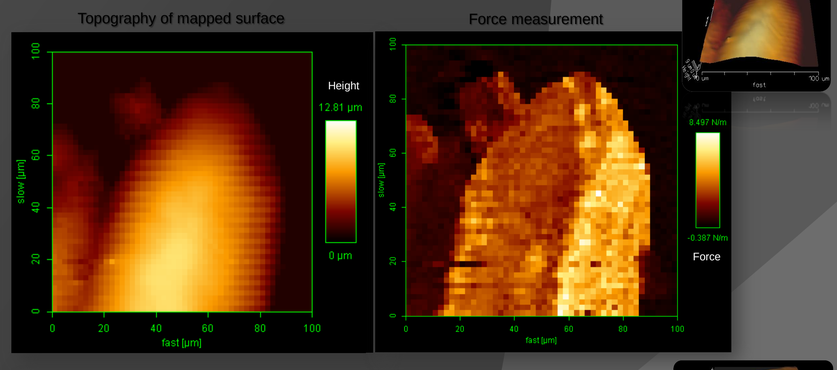


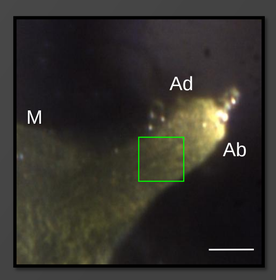


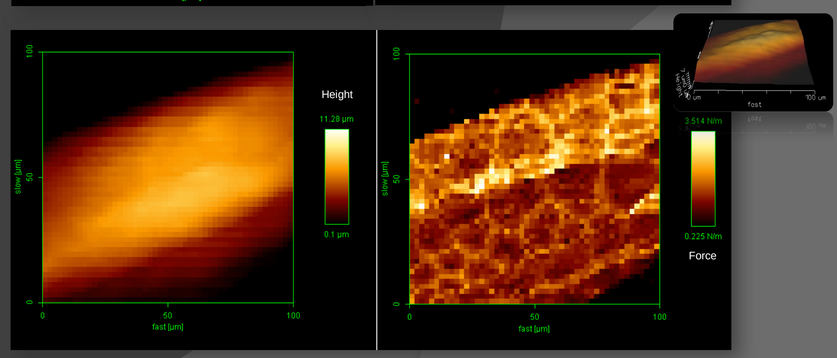
- Differences in cell wall extensibility can be measured and mapped using AFM with great resolution showing spatial heterogeneity of extensibility at cellular and tissue levels.

Tobacco primodia









➔ Cell wall is more extensible at leaf abaxial side (ab)

Future plan:

- Identifying expansins involve in leaf development from expression studies
- Testing the function of expansin through inducible amiRNA silencing approach and viscoelastic leaf growth model

References

[1]Cosgrove, D. J. 2000 Loosening of plant cell walls by expansins. *Nature* **407**, 321-326.
[2]Sampedro, J. & Cosgrove, D. J. 2005 The expansin superfamily. *Genome Biology* **6**, 242-250**.**
[3]Fleming, A. J., McQueen-Mason, S., Mandel, T. & Kuhlemeier, C. 1997 Induction of leaf primordia by the cell wall protein expansin. *Science* **276**, 1415-1418.
[4]Pien, S., Wyrzykowska, J., McQueen-Mason, S., Smart, C. & Fleming, A. 2001 Local expression of expansin induces the entire process of leaf development and modifies leaf shape. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 11812-11817
[5]Donnelly, P. M., Bonetta, D., Tsukaya, H., Dengler, R. E. & Dengler, N. G. 1999 Cell cycling and cell enlargement in developing leaves of Arabidopsis. *Developmental Biology* **215**, 407-419.