

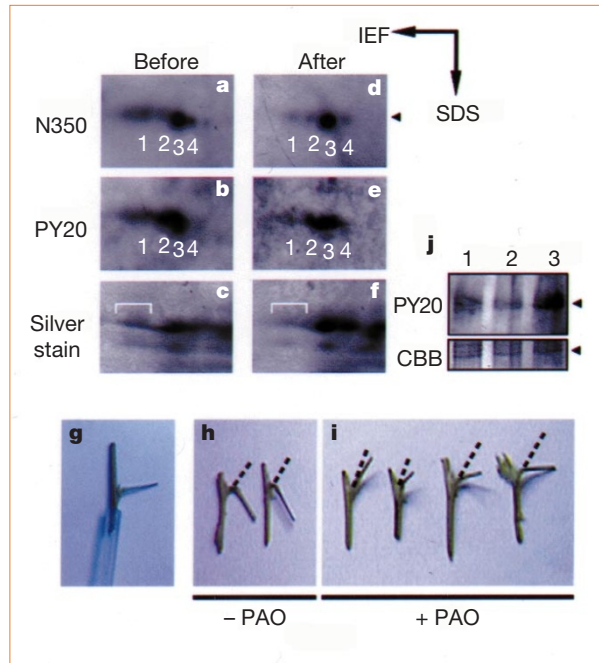
# Tyrosine phosphorylation in plant bending

Puckering in a ticklish plant is controlled by dephosphorylation of its actin.

The phosphorylation of the amino acid tyrosine in animal proteins acts as an on-off switch in numerous pathways that regulate growth, differentiation and oncogenesis<sup>1,2</sup>. In the unicellular slime mould *Dictyostelium*, tyrosine-phosphorylation of actin controls cell-shape changes and spore activity<sup>3,4</sup>, but the significance of protein-tyrosine phosphorylation in plants is unknown<sup>5</sup>. Here we show that actin in the contact-sensitive plant *Mimosa pudica* L. is heavily tyrosine-phosphorylated and that changes in the extent of phosphorylation correlate with the degree of bending of the plant's petioles. We propose that tyrosine-phosphorylation of actin controls movements in plants.

*M. pudica* closes its leaves and points its petiole downwards when touched (Fig. 1a,b). Petiole bending is caused by a rapid shrinking of the lower side of motor organs called the main pulvinus<sup>6-8</sup> (Fig. 1c,d). Cytochalasin B and phalloidin, which both have inhibitory effects on the actin cytoskeleton, inhibit the bending of the petiole, suggesting that actin is involved in bending<sup>9</sup>. We found that actin filaments in the motor cells at the lower side of the pulvinus, but not at the upper side, become more peripheral after bending (data not shown), although it is not known what molecular mechanism regulates these changes.

We carried out two-dimensional polyacrylamide gel electrophoresis on total proteins from the whole pulvinus before and after bending, and immunoblotted them with the anti-actin antibody N350 and reprobed with the anti-phosphotyrosine antibody PY20. Densitometry indicated that, before movement, about 80% of actin



**Figure 2** Correlation between the amount of actin tyrosine-phosphorylation and bowing in *M. pudica*. **a-f**, Total proteins from the whole pulvinus before (**a-c**) and after (**d-f**) petiole bending were immunoblotted with the anti-actin antibody N350 (**a,d**) or anti-phosphotyrosine antibody PY20 (**b,e**), or were silver-stained (**c,f**) after two-dimensional polyacrylamide gel electrophoresis (IEF; isoelectric focusing; SDS, denaturing polyacrylamide gel). Four actin spots were detected (numbered) (spots 1-3 are tyrosine-phosphorylated, spot 4 is not. **g-j**, Phosphorylation of actin from petioles before the plant is touched (**g** and **j**, lane 1), and after touching (**h,i**) in the absence (**h** and **j**, lane 2) or presence (**i** and **j**, lane 3) of phenylarsine oxide (PAO), a protein-tyrosine phosphatase inhibitor. Dotted lines indicate the original position of the petiole; arrowheads, actin bands.

molecules in the pulvinus were tyrosine-phosphorylated (Fig. 2a,b). After movement, the total amount of actin did not change, but immunoblots with N350 and PY20 on one-dimensional polyacrylamide gel electrophoresis showed that the amount of tyrosine phosphorylation in actin had dropped (data not shown).

Consistent with this decrease in tyrosine phosphorylation, we found that phosphorylated actin in spot 1, the most acidic isoform, disappeared (Fig. 2d-f) — probably because it had been dephosphorylated and shifted to a more basic isoform as a result of bending. In contrast, actin molecules in the stem, which does not move, were heavily tyrosine-phosphorylated, and the level of phosphorylation did not change during movement (data not shown). Changes in the levels of tyrosine phosphorylation of actin in the pulvinus therefore coincide with petiole bending.

In *Dictyostelium* spores, a decrease in actin phosphorylation is a prerequisite for recovering the dynamic actin cytoskeleton involved in spore germination. A specific inhibitor of protein-tyrosine phosphatases, phenylarsine oxide, inhibits the dephosphorylation of actin and the restoration of cell motility during germination<sup>4</sup>. We investigated the effects of phenylarsine oxide on *M. pudica*. When touched, the average bending angle of petioles treated with phenylarsine oxide (Fig. 2g-i) was smaller ( $37.3 \pm 20.24$  degrees;  $n = 25$ ) than that of controls ( $99.0 \pm 4.24$  degrees). There was no

decrease in the amount of actin phosphorylation following stimulation for the pulvinus exposed to phenylarsine oxide (Fig. 2j).

The bending of *M. pudica* is therefore correlated with reduced actin phosphorylation in the pulvinus. We propose that actin phosphorylation is essential for petiole bending resulting from actin cytoskeletal alterations. Actin may also be phosphorylated during the opening and closing of guard cells, as actin is dynamically arranged in these cells<sup>10</sup>.

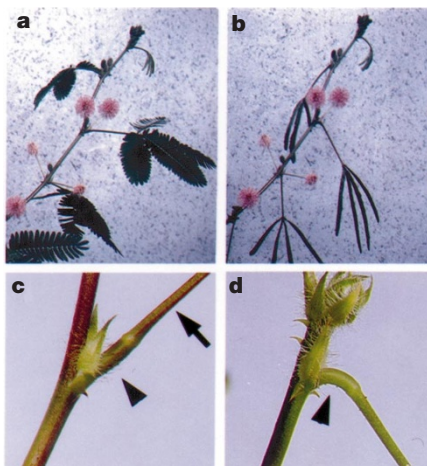
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**Figure 1** *Mimosa pudica* L. responding to stimulation by touch. **a,c**, Plant before touching and **b,d**, after touching; **c,d**, shows the main pulvinus (arrowheads), with a petiole (arrow)