Results

Part I

Analysis of aboveground organ-dependent production of XSP30 in cucumber roots

Expression of *XSP30* changes with plant maturity

Northern blot analyses of total RNA prepared from the roots of cucumber seedlings of different maturities demonstrated that expression of *XSP30* was barely detectable in the roots of 4-day-old seedlings, but increased gradually with maturity, concurrent with leaf development (Fig. 1), after 6 days. In contrast, a gene for a glycine-rich protein, *CRGRP-2*, that is present in cucumber xylem sap (Sakuta *et al.* 1998), was expressed at a measurable level in the roots of 4-day-old seedlings. While expression levels of *CRGRP-2* increased with age, a plateau was reached at the 14th day.

Diurnal oscillation of *XSP30* expression and protein production

Gel blot analysis of RNA prepared from the roots of cucumber seedlings grown under a 16 h light/8 h dark photoperiod showed a diurnal oscillation of expression of the *XSP30* gene (Fig. 2, A and B). The expression began to rise at midday and decline at dusk. The amplitude of this oscillation gradually increased from the 11th to 14th day. In contrast, the expression of the *CRGRP-2* gene showed only slight variation over the same period.

The amount of XSP30 protein in root xylem sap was evaluated by immunoblotting with XSP30 antiserum. The XSP30 protein level started to increase in the afternoon, reached a maximum at dusk, and then decreased during the night (Fig. 3A), in a pattern similar to that of *XSP30* gene expression. These results demonstrated a tight coupling between *XSP30* gene expression and XSP30 protein production.

When seedlings grown under a 16 h light/8 h dark photoperiod for 13 days were transferred to continuous light or continuous dark, *XSP30* expression showed the normal oscillation for two cycles. After two cycles, gene expression declined under continuous dark conditions, or showed an abnormal pattern of expression under continuous light (Fig. 4A). Expression of *CRGRP-2* did not change substantially under continuous light or dark (Fig. 4B).

Expression of *XSP30* depends on the presence of an intact shoot

When the aboveground tissues were removed from 15-day-old cucumber seedlings at the middle of the hypocotyl, *XSP30* expression increased transiently after 12 h and subsequently diminished (Fig. 5A), whereas *CRGRP-2* expression did not change (Fig. 5B). These results suggest that *XSP30* expression in cucumber roots is controlled by the presence of the aboveground organs.

Involvement of the first leaf in diurnal gene expression

Gene expression in roots was examined following removal of either the first leaf, or the cotyledons and shoot apex, from 13-day-old seedlings. Although *XSP30* expression in plants did not change when the cotyledon plus shoot apex were removed, gene expression was altered in plants from which the first leaf was removed. Most noticeably, the pattern of oscillation of gene expression declined after three cycles, but recovered from the fourth cycle, when the second leaf became fully expanded (Fig. 6A). No similar pattern was observed for *CRGRP-2* expression (Fig. 6B). These results

suggest that mature leaves, including the first and second leaves, are involved in upregulation of the diurnal expression of *XSP30* in cucumber roots, perhaps through activation of some translocatable signal.

Oscillating *XSP30* expression is related to leaf gibberellins

In order to examine the relationship between leaves and gene expression in the roots, GA_3 was sprayed on the cotyledon and shoot apex of cucumber seedlings following removal of the first leaf. Treatment with GA_3 fully restored the oscillating expression of *XSP30* in the roots (Fig. 7A).

To clarify the involvement of gibberellin in activating *XSP30* expression, uniconazole-P, an inhibitor of gibberellin biosynthesis, was applied to the shoots of intact plants. This resulted in a decrease in *XSP30* expression after 2 days (Fig. 8A). The simultaneous application of GA_3 with uniconazole-P resulted in complete recovery of the oscillation pattern (Fig. 8A). Furthermore, in order to elucidate whether gibberellin transported from the shoot directly controls the expression of *XSP30* in the root, 10^{-4} M GA_3 was supplied to the roots of uniconazole-P-treated plants. The application of GA_3 to roots did not restore the effect of uniconazole-P (Fig. 9A).

These results clearly indicated that gibberellin produced in the leaves indirectly controls the amplitude of the diurnally oscillating pattern of *XSP30* gene expression in the roots.