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RESEARCH PAPER

Light can rescue auxin-dependent synchrony of cell division in a tobacco cell line

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Abstract

Pattern formation in plants has to cope with ambient variability and therefore must integrate environmental cues such as light. Synchrony of cell divisions was previously observed in cell files of tobacco suspension cultures, which represents a simple case of pattern formation. To develop cellular approaches for light-dependent patterning, light-responsive tobacco cell lines were screened from the cell line *Nicotiana tabacum* L. cv. Virginia Bright Italia 0 (VBI-0). The light responsive and auxin-autonomous cell line VBI-3 was isolated. As in the progenitor line VBI-0, cell divisions are synchronized in VBI-3 during exponential growth phase. This synchrony can be inhibited by 1-*N*-naphthylphthalamic acid, an auxin transport inhibitor, and this process was accompanied by the disassembly of actin filaments. However, the synchrony could be rescued when the cells were cultured under white light or with exogenous indolyl-3-acetic acid. The rescue was most efficient for continuous far-red light followed by continuous blue light, whereas continuous red light was least effective. These findings are discussed in the context of phytochrome-induced auxin biosynthesis and auxin-dependent synchrony of cell division.

Key words: Auxin transport, cell division, NPA, phytochrome, synchrony, tobacco VBI-3.

Introduction

To exploit the evolutionary advantage of multicellularity, the organism has to assign different functions to the individual cells. In the framework of the open morphogenesis characteristic for plants, this cell differentiation is not achieved by a stereotypic cell lineage, but by cell–cell communication (for instance, Van den Berg *et al.*, 1995). Cormophytic land plants are constructed from modular elements, the telomes, that are organized around vasculature differentiated from parenchymatic cells. Fossile record shows that the pattern of vascular differentiation has been under the control of a polar flux of auxin for at least 375 million years (Rothwell and Lev-Yadun, 2005). In tissues such as root meristems, the differentiation of newly added elements is determined by coordinative signalling from preceding elements, i.e. the pattern is perpetuated in an iterative manner (leaf primordia, Reinhardt *et al.*, 2003; stem cells in the root, Van den Berg *et al.*, 1995).

It has previously been shown that suspension cultures of tobacco are endowed with a very simple form of pattern formation (Campanoni *et al.*, 2003). The culture cycle in these lines usually originates from unicellular stages and proceeds through a series of axial cell divisions to produce cell files that are endowed with a clear axis and, in most cases, with a clear polarity (Campanoni *et al.*, 2003). It has been found that cell division in these pluricellular files is partially synchronized, resulting in a higher frequency of cell files with even cell numbers than of files with uneven cell numbers. This synchrony of cell division can be perturbed by 1-*N*-naphthylphthalamic acid (NPA), an inhibitor of auxin transport (for a review, see Morris, 2000). This suggests that synchrony is under the control of polar auxin flux that coordinates the cell cycle of neighbouring cells (Nick, 2006).

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; IAA, indolyl-3-acetic acid; NAA, 1-naphthaleneacetic acid; NPA, 1-*N*-naphthylphthalamic acid.

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In a tobacco [*Nicotiana tabacum* L. cv. Bright-Yellow 2 (BY-2)] cell line, where actin was bundled in response to overexpression of yellow fluorescent protein (YFP)–talin, the synchrony of cell division was impaired, but when the actin bundles were detached into finer strands by addition of indolyl-3-acetic acid (IAA) and, to a reduced extent, by 1-naphthaleneacetic acid (NAA), the synchrony was restored (Maisch and Nick, 2007). These observations are consistent with published reports on actin-dependent transport of PIN proteins that are generally discussed as markers for auxin efflux (for reviews, see Muday and Murphy, 2002; Blakeslee *et al.*, 2005). However, this presumed link between actin organization and auxin transport has been questioned recently by experiments where PIN1 and PIN2 maintained their polar localization, although actin filaments had been eliminated by the artificial auxin 2,4-dichlorophenoxy-acetic acid (2,4-D), or the phytotropin NPA (Rahman *et al.*, 2007). Thus, although actin seems to play a role in the polarity of auxin fluxes, the relationship is, first, not simple, and, secondly, far from being understood.

The molecular components that participate in the communication between actin and auxin remain mostly unknown. Generally, the nucleation of actin filaments is controlled by several, partially directly actin-associated, protein complexes including the Rho-related GTPases of plants (ROPs), the WAVE (for Wiskott–Aldrich syndrome protein family verproline homologous) complex, and the actin-related protein (ARP) 2/3 complex. These regulators modulate the actin cytoskeleton through an elaborate signalling network (for a review, see Xu and Scheres, 2005). In fact, dual fluorescence visualization of actin and ARP3 in BY-2 cells demonstrated a graded distribution of ARP3 in the terminal cells of a file, and this gradient persisted when the file disintegrated into single cells, whereas the asymmetric distribution of the auxin efflux marker PIN1 was lost and had to be re-established during the early phase of the new culture cycle (Maisch *et al.*, 2009). These observations indicate that actin nucleation might be upstream of the events that culminate in a polar distribution of auxin efflux carriers.

Due to the photosynthetic lifestyle of plants, the most relevant environmental signal for plant development is light. The classical system to study light-dependent pattern formation has been the anthocyanin pattern in mustard cotyledons that is under control of the phytochrome photoreceptor system (Mohr, 1972). A cellular analysis of this patterning process revealed that the response of individual cells was very stochastic (Nick *et al.*, 1993), with expression of a complete response in some cells co-existing with the complete absence of a response in even the neighbouring cells. These all-or-none-type differences even of adjacent cells were observed already for the early stages of the response (transcription of chalcone synthase, a key enzyme for flavonoid synthesis). These responses were later coordinated and integrated over the entire organ. The patterns observed after microirradiation of different regions of the cotyledon could be explained by a working model, where the cellular responses were regulated by directional signals travelling in the apicopetal direction along the leaf veins. However, the nature of these signals could not be revealed, which is due to certain limitations of this experimental system: mustard is very recalcitrant to transformation, and the very small cells of the young cotyledons are not very amenable to cell-biological analysis. This stimulated the search for alternative systems to study pattern formation in plants. Tobacco suspension cultures with their patterned division response would be accessible to both genetic engineering and cell-biological analysis. However, they are not responsive to light, the most relevant environmental signal in plant life.

To overcome the drawback of the tobacco system, light-responsive lines of tobacco have been searched. Success was achieved in isolating a derivative of the line VBI-0 that responds to light. This line, VBI-3, has preserved the patterning system of its progenitor line, and this pattern is sensitive to inhibition of polar auxin flux by NPA. However, this pattern has acquired independence from exogenous auxin. The inhibition of division synchrony by NPA can be rescued by addition of exogenous IAA and by light. The wavelength dependence of this rescue indicates that the light response is triggered by photolabile phytochrome (phyA). This is the first time, to our knowledge, that light-dependent patterning has been shown in a plant cell culture.

Materials and methods

Cell line

The tobacco (*N. tabacum* L. cv. 'Virginia Bright Italia') cell line VBI-3 was selected as the clonal line from the cell line VBI-0 (Opatrný and Opatrná, 1976) by its ability to grow in the absence of exogenous auxins. Auxin-dependent VBI-0 cells were grown in slightly modified Heller's liquid medium (Heller, 1953) in the presence of the synthetic auxins 2,4-D (4.5 μM, Sigma-Aldrich, Deisenhofen, Germany) and NAA (5.4 μM, Sigma-Aldrich). To obtain auxin-autonomous lines, VBI-0 cells were selected in liquid medium without supplemental auxin over two successive subculture intervals until all files had completely disintegrated into individual cells. This suspension was then plated on solid auxin-free medium in Petri dishes and cultivated in continuous darkness at 25 °C. After 5 weeks, small microcolonies were transferred to fresh auxin-free medium and cultured in continuous light. Cells were subcultured every 3 weeks, inoculating 3 ml of stationary cells into 30 ml of fresh medium in 100 ml Erlenmeyer flasks, and incubated at 25 °C on an orbital shaker (KS250 basic, IKA Labortechnik, Staufen, Germany) at 150 rpm (18 mm diameter) in the dark.

NPA and auxin treatments

Sterile-filtrated NPA was added at inoculation from a stock of 50 mM in dimethylsulphoxide (DMSO) to a final concentration of 5 μM. For auxin treatment, either IAA (final concentration 2 μM) or a combination of 2,4-D (final concentration 4.5 μM) and NAA (final concentration 5.4 μM) was added at inoculation after sterile filtration. Equal volumes of sterile DMSO and ethanol, respectively, were added to the control samples to account for possible effects of the solvent.

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Quantification of pattern and morphology

From each sample, 0.5 ml aliquots of cells were collected through the logarithmic culture phase (days 7, 8, 9, 10, and 11 after inoculation) and immediately viewed under an AxioImager Z.1 microscope (Zeiss, Jena, Germany). Frequency distributions over the number of cells per individual file were constructed from 200 individual cell files (containing up to 10 cells per file) for each sampling day. The data from the 5 d in two independent experimental series were pooled to construct frequency distributions over cell number per file, thus representing 2000 individual cell files. Cell densities were determined by using a Fuchs–Rosenthal haematocytometer under bright-field illumination.

Light sources and irradiation

To assess the wavelength dependency of the light effect on patterning, the cells were cultivated in suspension under the conditions described above with continuous white light, red light (λ_{max} 650 nm), far-red light (λ_{max} 735 nm), and blue light (λ_{max} 470 nm) adjusted to a fluence rate of 26.0 μmol m^{-2} s^{-1}.

The white light was obtained from cool white fluorescent bulbs (Atlanta Light Bulbs Inc., OSRAML18W/25 UNIVERSAL WHI). Blue light (Avago Technologies, HLMP-HB57-LP000), red light (Vishay, TLDR5800), and far-red light (Quantum Devices, QDDH 73502) were obtained with LED arrays. The light intensities were measured by a quantum sensor (Skye Instrument Ltd, SKP 215) except far-red light which was measured by a custom-built photometer with a photodiode sensor.

Visualization of actin microfilaments

Actin microfilaments were visualized as described in Maisch and Nick (2007). Suspended cells were fixed for 10 min in 1.8% (w/v) fresh paraformaldehyde in standard buffer (0.1 M PIPES, pH 7.0, supplemented with 5 mM $MgCl_2$ and 10 mM EGTA). After a subsequent 10 min fixation in standard buffer containing 1% (v/v) glycerol, cells were rinsed twice for 10 min with standard buffer. Then, 0.5 ml of the resuspended cells were incubated for 35 min with 0.5 ml of 0.66 μM fluorescein isothiocyanate (FITC)–phalloidin (Sigma-Aldrich) prepared freshly from a 6.6 μM stock solution in 96% (w/v) ethanol by dilution (1:10, v/v) with phosphate-buffered saline (PBS; 0.15 M NaCl, 2.7 mM KCl, 1.2 mM KH_2PO_4, and 6.5 mM Na_2HPO_4, pH 7.2). Cells were then washed three times for 10 min in PBS and observed immediately by confocal laser scanning microscopy (TCS SP1; Leica, Bensheim, Germany) using the fixed-stage configuration of the confocal laser scanning microscope with the 488 nm laser line of the ArKr laser and a four-frame averaging protocol.

Results

The pattern of cell division within the cell files of VBI-3

The tobacco cell line cv. Virginia Bright Italia 3 (VBI-3) has been derived from the tobacco cell line VBI-0 (Opatrný and Opatrná, 1976) as a clonal line selected for auxin-autonomous growth in continuous light. VBI-0 and VBI-3 are similar with respect to their life cycle, cell file axiality, and cell division synchrony (Fig. 1). The growth curves (Fig. 1A), plotted as cell density relative to the final cell density against time, are virtually identical for VBI-3 and VBI-0. As observed in the ancestral VBI-0, cell division in VBI-3 exhibits a clear axiality (Fig. 1B) and initiates from single cells and two-cell files (Fig. 1C) to generate pluricellular cell files. Frequency distributions constructed over the number of cells per file show higher frequencies of files with even cell

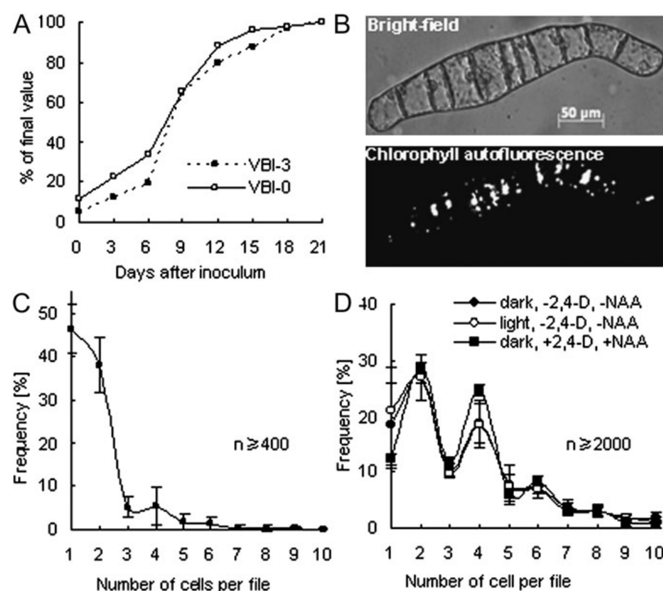

Fig. 1. Cell division in VBI-3 follows a pattern that is independent of exogenous auxins. (A) Cell density of VBI-3 and VBI-0 over time after subcultivation. Density is given relative to the final value after 21 d of cultivation. (B) Morphology of VBI-3; bright-field and chlorophyll autofluorescence. (C) Frequency distribution over the number of cells per file in the initial state (day 0). (D) Frequency distribution during the logarithmic phase in the presence of exogenous auxins (control) or in the absence of NAA and 2,4-D for cultivation in the dark or under white light. The distributions are based on ⩾400 (C) or ⩾2000 (D) cell files from two independent experimental series. Error bars indicate the SE.

numbers as compared with files with uneven cell numbers (Fig. 1D) as described for the ancestral line VBI-0 (Campanoni *et al.*, 2003). However, in contrast to VBI-0, where cell division strictly requires exogenous NAA and 2,4-D (data not shown), cell division and synchrony in VBI-3 are independent of NAA and 2,4-D. In contrast to the ancestral VBI-0, VBI-3 forms chlorophyll-containing plastids when cultured under light conditions as detected by chlorophyll autofluorescence (Fig. 1B). However, the synchrony of cell division is not significantly altered when VBI-3 files cultivated in the dark are compared with those that have been cultivated under white light (Fig. 1D).

The synchrony of cell division is affected by NPA and can be rescued by light or IAA

To investigate whether the division synchrony (monitored as predominance of even-numbered cell files) was dependent on auxin transport, NPA, an inhibitor of polar auxin, was added to the medium at subcultivation either in the dark (Fig. 2A) or under white light (Fig. 2B). In the dark, this treatment affected the frequency distribution by decreasing the frequency of files with four and six cells (Fig. 2A). As a consequence, the distinct frequency peak at four cells disappeared, i.e. the synchronization of cell division was affected. When the cells were cultured under white light, the frequency of files with four cells was maintained at the level

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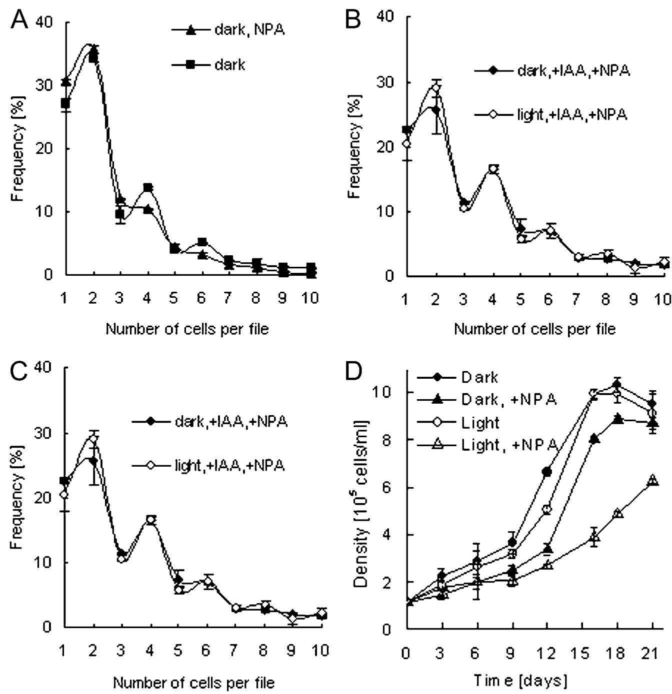

Fig. 2. The effect of NPA on the division pattern can be rescued by light or exogenous IAA. Frequency distributions of cell number per file are shown for cells cultivated with 5 μM NPA, either in the dark (A) or under white light (B), or after treatment with 2 μM IAA (C). Control cells in A and B have been cultivated in the absence of NPA. Each distribution is based on ⩾2000 cell files from two independent experimental series. (D) Cell density over time after subcultivation of dark- and light-grown cultures in the absence or the presence of 5 μM NPA. Error bars indicate the SE.

observed in the absence of NPA, i.e. the impaired synchrony in response to NPA was rescued by light (Fig. 2B). To exclude that this rescue was mimicked by a photo-inactivation of NPA, a control experiment was performed where the medium complemented with NPA was irradiated under the same experimental light for 11 d and then used for subcultivation. This pre-irradiated medium caused the same inhibition of synchrony as medium freshly complemented with NPA (data not shown). Similar to white light, exogenous IAA could overcome the effect of NPA and restore the predominance of files with four cells over those with three or five cells (Fig. 2C). In combination with white light, exogenous IAA could even overcome the effect of NPA completely; such that a combination of white light, exogenous IAA, and NPA produced the same distribution as observed for a cell line that had been cultivated in the absence of NPA (compare Fig. 2C and B).

The rescue of the division synchrony by light depends on light quality

Since irradiation with white light reversed the effect of NPA on the division synchrony, an experiment was performed to determine whether this reversal was dependent on light quality (Fig. 3). When the cells were cultured under continuous red light, only a very weak recovery of the pattern was observed (Fig. 3A). This recovery was more significant for continuous blue light, especially for files with <5 cells, but it was incomplete for longer files (Fig. 3B). However, for irradiation with continuous far-red light, the frequency of cells with four and six cells was clearly elevated (Fig. 3C) and the resulting frequency distribution became congruent with that observed in the absence of NPA (compare Figs 3C and 2B). Thus, continuous far-red light can overcome the effect of NPA on synchrony.

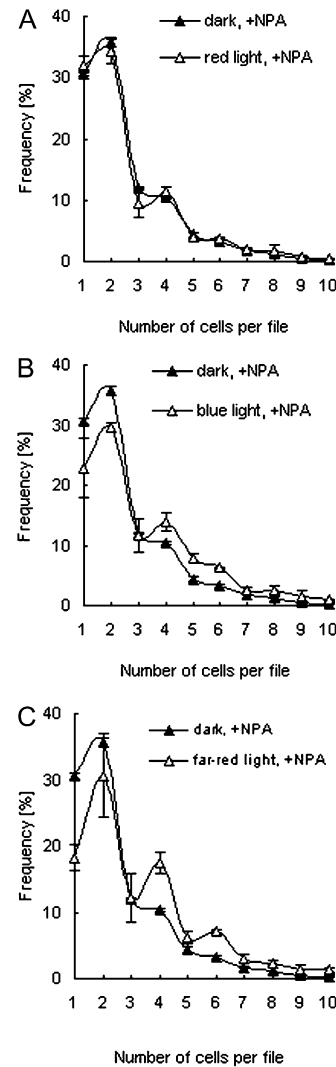

Fig. 3. The light-dependent rescue of the division pattern in the presence of NPA depends on light quality. Frequency distributions of cell number per file were constructed for incubation with 5 μM NPA under equal fluence rates (26.0 μmol m^{-2} s^{-1}) of continuous red (A), blue (B), or far-red light (C). Each distribution is based on ⩾2000 cell files from two independent experimental series. Error bars indicate the SE.

NPA affects the organization of actin filaments

Since division synchrony has been shown to depend on actin organization (Maisch and Nick, 2007), and the effect of certain phytotropins on auxin transport has been ascribed to the induction of actin bundles (Dhonukshe

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et al., 2008), the response of actin filaments to NPA in VBI-3 was followed. Actin filaments were visualized by fluorescent phalloidin in combination with a mild fixation protocol after 2, 16, and 24 h of incubation with 5 μM NPA. The response of actin organization to NPA over time was followed by confocal microscopy. In the absence of NPA [but in the presence of the solvent used for NPA (DMSO) at the same concentration as in the treated samples], trans-vacuolar actin strands reach from the nucleus into the cell periphery and diffuse into a cortical actin array (Fig. 4A). At 2 h after addition of NPA, this cortical actin array as well as the transvacuolar strands have significantly faded, whereas the perinuclear actin array has become more prominent (Fig. 4B). This repartitioning of actin towards the nucleus leads to a situation where only a few bundles of actin emanate from the nucleus at 16 h after addition of NPA (Fig. 4C) and eventually actin disintegrates into short fragments (Fig. 4D).

Discussion

As a consequence of their sessile lifestyle, plant development has to respond to environmental cues, with light being one of the most central signals. The open morphogenesis characteristic for plants requires that pattern formation must cope with the continuous expansion of the *Bauplan* through reiterative addition of new elements (for a review, see Nick, 2006). In previous work, tobacco cell lines such as VBI-0 (Campanoni *et al.*, 2003) or BY-2 (Maisch and Nick, 2007) have been used as simple systems to study cellular aspects of reiterative pattern formation. It can be shown that, in these lines, cell division is synchronized by a polar flux of auxin, and that this synchrony is disrupted either by inhibiting auxin efflux through NPA or by inducing bundling of actin filaments through overexpression of the actin-bundling protein talin. A drawback of this experimental system has been the lack of a light response in these cell lines. Clonal descendants of the VBI-0 line were therefore screened for auxin-autonomous growth and responsiveness to light manifested by chlorophyll synthesis. The cell line VBI-3, isolated from this screen, has conserved the division pattern characteristic of its ancestral line VBI-0. For instance, cell division follows a similar time course, and the predominance of even-numbered files as a manifestation of synchronized cell division is maintained (Fig. 1). However, in contrast to the ancestral line VBI-0, both division activity and division synchrony are independent of exogenous auxin in VBI-3. The synchrony of division can be disrupted by NPA, suggesting that polar auxin transport is responsible for the division synchrony (Fig. 2). When time courses of cell density in the dark versus the light in the absence or presence of NPA (Fig. 2D) were scored, it was observed, as expected, that cell division progresses somewhat more slowly after treatment with NPA. However, there was an interesting difference between dark and light: in the dark, NPA caused only a minor delay, and the culture reached the same final density of cell files (NB: the average number of cells per individual file was basically unchanged). In the light, the delay was much more pronounced (again, the average number of cells per individual file was not much changed). The division synchrony can be restored by light or by exogenous IAA. When the light effect was analysed further by administering different light qualities at equal fluence rates (Fig. 3), continuous far-red light was found to rescue the synchrony most efficiently, followed by blue light, whereas red light was least effective.

Among the plant photoreceptors, only the phytochromes are able to sense far-red light. Plant phytochromes are synthesized in the inactive Pr form and, upon exposure to light, are transformed into the active Pfr form. Since Pr absorbs red light and Pfr preferentially absorbs far-red light, it would be expected that phytochrome-triggered responses are induced by red light (establishing a high photoequilibrium of active Pfr in relation to total phytochrome) and inactivated by far-red light. In fact, this has been the operational criterion to define a phytochrome-dependent light response (Butler *et al.*, 1959). Phytochrome is encoded by a small gene family with five members in *Arabidopsis* or three members in rice. One member,

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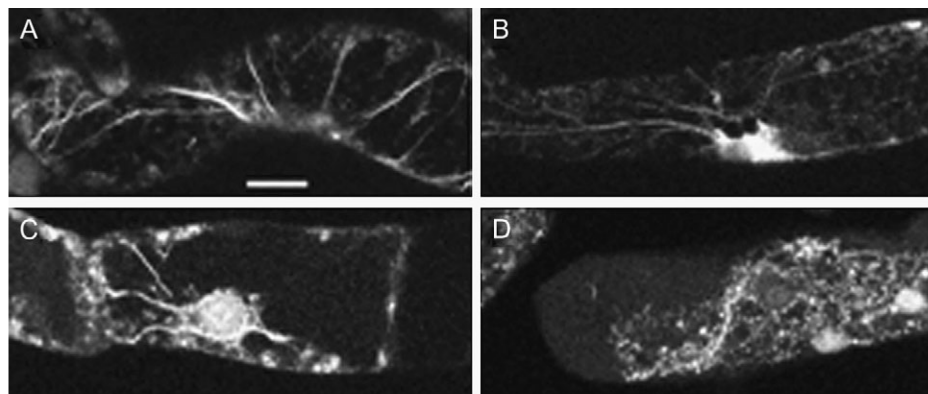

Fig. 4. Effect of NPA on actin organization in VBI-3 cells after incubation for 2 h (B), 16 h (C), and 24 h (D). The control (A) was treated with DMSO. NPA at 5 μM was added to the medium in the presence of NAA and 2,4-D when subculturing. The cells were cultured in the dark. Projections from z-stacks through the entire cell are shown. Scale bar=20 μm.

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so-called phyA, decays upon irradiation, whereas the other members (phyB–E) are synthesized constitutively, and do not decay upon irradiation with light so that they are terminologically separated as 'stable' from the 'labile' phyA phytochromes (Furuya, 1993). The photodestruction of phyA has the consequence that continuous red light will be less effective in triggering a phyA-dependent response, because more phyA per unit time will be shifted into the Pfr than can be utilized for signalling. This excess phyA–Pfr will then undergo decay and therefore is not available for signalling. In contrast, continuous far-red light will only produce the phyA–Pfr necessary to trigger signalling, avoiding excess Pfr, such that the proportion of Pfr undergoing decay is negligible in relation to that channelled to signalling. Therefore, for continuous irradiation, far-red light will be more efficient than red light. This so-called far-red-dependent high irradiation response is specific for photolabile (phyA) species, but is not observed for responses triggered by the light-stable phytochromes (Kneissl *et al.*, 2008). A similar high irradiation response is expected for other light qualities that will establish a low photoequilibrium, for instance blue light. Therefore, phyA-dependent responses are expected to be most efficiently induced by continuous far-red light and blue light, whereas continuous red light should evoke only a weak effect. This is exactly what is observed for the light quality dependence of division synchrony in VBI-3 (Fig. 3), consistent with the hypothesis that the responsible photoreceptor is a photolabile (phyA) form of phytochrome. It cannot be excluded, however, that a blue light receptor (a cryptochrome) acts in concert with phyA. Bichromatic irradiation with blue light and far-red light establishing different photoequilibria would be required to exclude a cryptochrome activity. However, phyA as the exclusive photoreceptor would be sufficient to explain the observed wavelength dependency of synchrony rescue in VBI-3. This is not the first example of an interaction between NPA and light. A decade ago, Jensen *et al.* (1998) reported that NPA inhibited hypocotyl elongation in *Arabidopsis* light dependently. Interestingly, the wavelength dependency was the same as found for division synchrony in VBI-3: continuous far-red light and blue light were most effective, whereas continuous red light had little impact. However, in hypocotyls, NPA was not active in the dark, but requires light to be effective. In contrast, in patterned cell division, NPA is active in the dark, but its activity is alleviated by light. This difference may be related to the fact that hypocotyl elongation is under control of gibberellins, whereas gibberellins have been found not to be effective in suspension cultures of tobacco (Hofmanová *et al.*, 2008).

Since the synchrony of cell division depends on a polar flux of auxin (manifested as sensitivity of synchrony to the auxin transport inhibitor NPA), and since light can rescue synchrony in the presence of NPA, the light effect must be somehow related to auxin. It should be kept in mind, however, that the effect of auxin fluxes is modified, especially during the first cycles of a developing cell file, by the intrinsic polarity of the progenitor cell (Campanoni *et al.*, 2003), which becomes manifest, for instance, by the fact that the frequency of three-cell files after treatment with NPA is similar, but does not exceed the frequency for four-cell files. At present, two possible scenarios can be developed to explain the light effect. Light could stabilize the activity of the auxin efflux carrier against inhibition through NPA. From binding studies with different ligands, it has been inferred that the ligand-binding surface of this carrier is multifaceted (Brunn *et al.*, 1992). It is therefore conceivable that light-dependent factors bind to this surface, and induce conformational changes culminating in a reduced affinity of NPA for this carrier. Alternatively, light could shift the cycling of the auxin efflux carrier towards the active, membrane-bound state in a similar manner to its induction by IAA (Paciorek *et al.*, 2005). In response to NPA, the actin filaments in VBI-3 are partitioned towards the nucleus and eventually disintegrate (Fig. 4), a response which has also been reported for roots of *Arabidopsis* (Rahman *et al.*, 2007). Actin filaments participate in the synchronization of cell division in tobacco cell cultures by regulating polar auxin flux (Maisch and Nick, 2007), and, in turn, the organization of actin is regulated by auxin establishing a feedback loop between cellular auxin content, auxin flux, and actin organization. Light could interfere with this feedback loop by stabilizing actin filaments against the disruption through NPA. In fact, phytochrome activation has been shown to alter the organization of actin filaments in epidermal cells of Graminean coleoptiles (Waller and Nick, 1997; Waller *et al.*, 2002). Experiments are ongoing at present in an attempt to establish transgenic VBI-3 lines expressing the actin-binding domain of plant fimbrin in fusion with green fluorescent protein (GFP) as a tool to follow actin responses to light, auxin, and NPA *in vivo*.

However, there is a much more straightforward model to explain the light effect on division synchrony. The inhibition of division synchrony was alleviated not only by light, but also by exogenous IAA (Fig. 2C). This indicates that, upon inhibition of polar auxin transport, it is a decrease of the cellular IAA content which impairs efficient synchronization. Evidence from BY-2 cells expressing the auxin-responsive DR5 promotor driving a GFP reporter in combination with localized release of auxin demonstrates that the terminal cells of a file act as auxin sources that export auxin to their downstream neighbours; treatment with NPA causes a significant decrease of DR5 activity in those downstream cells, indicating that cell-autonomous synthesis is limiting (Kusaka *et al.*, 2009). If phyA would stimulate the synthesis of IAA, the constraints imposed upon synchronization through the application of NPA would be relieved in the same way as they are relieved when exogenous IAA is added directly. Consistent with this model, the recently published work by Tao *et al.* (2008) demonstrates that continuous activation of phyA by white light of a high red/far-red ratio (simulating canopy shading) can induce a novel aminotransferase, TAA1, that catalyses the formation of indole pyruvic acid from tryptophan as the first step of a previously proposed, but uncharacterized,

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auxin synthetic pathway. If this or a similar auxin synthesis pathway were activated by phyA in VBI-3, this could explain both why patterning becomes more resistant to NPA and why the light effect can be mimicked by exogenous IAA.

There is, however, an additional, spatial effect to be considered. When time courses of overall cell density were scored (Fig. 2D), NPA was observed to cause a general reduction of global cell division activity. This reduction was hardly visible in the dark, but was pronounced upon illumination. This result indicates that in the light not only synchrony, but cell division *per se* was much more dependent on auxin efflux from the terminal cell into the proximal cells of the file. In other words, auxin synthesis might be more strictly confined to the terminal cell in response to light- as compared with dark-grown cells. In the meantime an approach to test this exciting possibility directly by localized release of auxin from an esterase-resistant caged precursor has been established (Kusaka *et al.*, 2009).

Patterned cell division has been studied in great detail in the root meristem of *Arabidopsis*, where the pattern can be traced back to early embryogenesis. However, the meristematic pattern of cell division is already established when the root meristem becomes accessible to cell-biological inspection, and it is very difficult, if not impossible, to manipulate these patterns in a fundamental manner. Thus, root meristems represent a beautiful system to study pattern perpetuation, but for the analysis of pattern induction simpler systems that are less pre-determined might be more appropriate. Suspension lines of tobacco provide such models to study the primordial stages of division patterning and, in general, cellular aspects of cell division. A major drawback of the tobacco suspension system is the absence of a specific response of patterning to important environmental signals, such as light. The isolation of the VBI-3 cell line overcomes this drawback and allows the investigation of the cellular events that link photomorphogenesis to patterned cell division. To gain insight into the mechanisms driving light-dependent patterning, it is planned to follow the response of cellular auxin content to irradiation. Moreover, in order to be able to address the spatial pattern of auxin abundance in a cell file, transgenic lines that express the auxin-responsive DR5 promotor driving GFP as reporter are presently being generated. In parallel, a novel approach to control the concentration of IAA at cellular or even subcellular resolution with an esterase-resistant caged auxin that can be locally released by spatially confined irradiation has recently been established (Kusaka *et al.*, 2009), and this technique will be used to study patterning in the context of specific transcellular auxin gradients.

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