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Hypocotyl elongation is an early developmental process regulated antagonistically by light and auxin. To highlight the interaction between both signals, we studied the photoregulation of the auxin-induced tomato (Lycopersicon esculentum Mill.) gene LeEXT involved in this process. RNA gel blot analysis indicated that this gene is down-regulated in response to blue light. We demonstrate that this response is principally mediated by the blue light photoreceptor cry1, but an interaction with the red/far-red light photoreceptors phyA, phyB1 and phyB2 has also been established. Furthermore, the polar auxin transport inhibitor NPA reverts the blue light inhibition of Lycopersicon esculentum gene encoding xyloglucan endotransglycosylase (LeEXT) expression, when it has the opposite effect in the dark or under red light. These results provide strong support for a specific interaction between auxin and blue light transduction pathways in the control of LeEXT expression, and therefore, of hypocotyl elongation in tomato.

Abbreviations: cry1 – cryptochrome 1; dgt – diageotropica mutant; LeEXT – Lycopersicon esculentum gene

encoding xyloglucan endotransglycosylase; NPA – naphtylphtalamic acid ; phyA, B1, B2 – phytochrome A, B1, B2; WT – wild type

Introduction

Light contains a complex set of signals, influenced by neighbouring plants, non-biotic surroundings and the diurnal and seasonal cycles. These signals are monitored by photoreceptors to adapt plant growth and development to its environment. The light receptors, including the red (R) and far red (FR) receptors (phytochromes) and the blue (B) receptors (cryptochromes and phototropins) are relatively well characterised (Christie and Briggs 2001; Fankhauser 2001; Quail 2002), and many transduction elements are described (Bowler et al. 1994; Fankhauser and Chory 1999; Withelam and Halliday 1999; Nagy et al. 2001; Quail 2002). However, most of the light signalling pathways described are cell-autonomous and cannot account for the co-ordinated development of the whole plant. In some instances, the transmission within the plant of an information related to a light signal has been described; it is the case in Petunia, where the irradiation of leaves and sepals leads to the induction of anthocyanin biosynthesis in petals (Moscovici et al. 1996), or in potatoes, where tuberisation is controlled in the leaves at least by phytochrome B (phyB) (Jackson et al. 1998). Phytohormones have been proposed to be involved in the physiological transmission of light signals, but an unequivocal demonstration of such a role has been prevented by the paucity of direct molecular data. The most convincing results concern the invol- vement of cytokinins in the photoregulation of the kinase-encoding WPK4 gene of wheat (Sano and Youssefian 1994), the photoregulation of gibberellin metabolism (Kamiya and Garcia-Martinez 1999) and brassinosteroid biosynthesis (Kang et al. 2001), and the putative role of ABA in the phyto- chrome-related induction of the NPR1 gene of Lemna (Weatherwax et al. 1998). However, most of the recent work pointing out interactions between hormone and light signalling focuses on molecular interactions of transduction elements, highlighting the complex network of developmental regulations, but not on the physiological integration of the sig- nals at the whole plant level. The role of ARR4 in both transduction pathways of phyB and cytokinins is one example (Fankhauser 2002).

For many years, different approaches have been used to analyse the relationships between light and auxin in the control of development. Most studies focused on the antagonism between both factors in the regulation of cell elongation, using hypocotyl or coleoptile simple models. According to photo- biological experiments or studies of photomorpho- genic mutants, light signals would decrease auxin levels (Iino 1982; Kraepiel et al. 1995). In other cases, light would control auxin sensitivity (Jones et al. 1991) or auxin localisation (Jones et al. 1991; Behringer and Davies 1992). These latter results suggest a very attractive hypothesis, where a polar auxin transport would occur from the apex to the roots without stimulating the stem growth under light conditions, whereas in darkness, auxin would accumulate in the outer layers of the stems thus inducing a high elongation rate. More recently, conflicting data occurred concerning the photore- gulation of auxin transport. Jensen et al. (1998) demonstrated that the inhibition of hypocotyl elon- gation in Arabidopsis by the auxin-transport inhibitor naphtylphtalamic acid (NPA) was limited to light conditions, and that the high elongation rate of etiolated seedlings was not auxin-dependent.

These data suggest that the growth inhibition trig- gered by a dark–light transition does not involve a modification of auxin transport. In the control of hypocotyl elongation in tomato, we described an opposite switch from an auxin-dependent in the dark to an auxin-independent process in the light (Kraepiel et al. 2001). However, Shinkle et al. (1998) have demonstrated, using dim-red-light, that a dark-light transition increases the intensity and velocity of polar auxin transport in cucumber hypocotyls, in correlation to the growth rate inhibition. From all these data, many correlations have been established between the light-induced modifi- cation of auxin physiology and the photomorpho- genic processes, but detailed causality-relationships have not been demonstrated.

Recent genetic and molecular data seem to con- firm the role of auxin in different aspects of photomorphogenesis. Several Arabidopsis mutants have been identified as impaired in their responses to light and the corresponding mutated genes belong to the early auxin-regulated gene families, which are involved in the auxin responses in plants (Reed 2001). This is the case of the GH3-like genes identi- fied in the dfl1 and fin219 mutants, which are defective in their responses to all light qualities and to far-red light, respectively (Hsieh et al. 2000; Nakasawa et al. 2001). It is also the case of AUX/IAA genes, such as the IAA3 gene mutated in the shy2 mutants (Kim et al. 1996, 1998; Reed et al. 1998; Tian and Reed 1999; Tian et al. 2002). These mutants have been identified as suppressors of the hy2 chromophore and the phytochrome B muta- tions for several photomorphogenesis-related phe- notypes. The phosphorylation by phytochrome of the transcription factors belonging to the IAA/ AUX family could be a major molecular process involved in the interaction of phytochromes and auxin transduction pathways (Colon-Carmona 2000). Another interaction between light and auxin may take place at the level of auxin transport as revealed by the allelic mutants doc1 and tri3 identi- fied as light-response and auxin transport mutants, respectively (Gil et al. 2001). Moreover, the mole- cular studies of the role of the HD-Zip protein ATHB-2 demonstrated its involvement both in the shade avoidance response and auxin transport (Steindler et al. 1999). Finally, recent DNA array technologies have allowed to identify a large num- ber of genes directly or indirectly regulated by light and auxin, including the early auxin-induced genes themselves as well as photosynthetic genes (Gil et al. 2001; Tian et al. 2002).

In this work we describe the photoregulation of the auxin-induced Lycopersicon esculentum gene encoding xyloglucan endotransglucosylase (LeEXT). This gene encodes a xyloglucan endo- transglycosylase, which is supposed to be involved in the regulation of the hypocotyl elongation pro- cess (Catala et al. 1997; Rose and Bennett 1999). Using an auxin-transport inhibitor and a mutation conferring auxin insensitivity, we analyse the role of auxin in the control by light of LeEXT expression.

Materials and methods

Plant materials and growth conditions

All the photomorphogenic mutants used were iso- lated in Wageningen (Kendrick et al. 1997; Weller et al. 2001) from the tomato MoneyMaker (MM) background. The aurea mutant is deficient in chro- mophore biosynthesis and supposed to be deficient in the functionality of all phytochromes. The fri^{1}, tri^{4} and $cry1^{1}$ mutants are null mutants of the PHYA, PHYB1 and CRY1 genes, respectively. The phyA–phyB1–phyB2 triple mutant (line 70F) has been isolated from gamma-ray mutagenised fri–tri seeds (Kerckhoffs et al. 1999). The

phyB2 mutation results in an unspliced second intron. The auxin-insensitive mutant diageotropica (dgt) is compared to its wild-type isogenic line Ailsa Craig (AC) (Coenen and Lomax 1998).

For RNA analysis, dry seeds were treated by 2.5% sodium hydrochloride for 30 min to facilitate the germination, abundantly rinsed and imbibed for 48 h at 4 C in imbibition buffer (KNO_3 5 mM, NaH_2PO_4 10 mM, K_2HPO_4 10 mM). Imbibed seeds were plated on a wet filter paper and put in growth chamber (22 C ± 1, 60% ± 10 relative humidity) in the dark. Each day, the ger- minated seeds (when the radicle has protruded the seed coat) were sown on moist sand (sable de Fontainebleau, Prolabo) on square-holed Petri dishes (100 seeds per 12 12 cm plate) under green safe light. The plates, daily moistened by distilled water, were kept in darkness for 6 days before the 24 h light treatments. The naphtylphtalamic acid (NPA) treatments were applied from 3 h before the end of the irradiation, wetting the plates by the aqueous solutions.

For length measurements of hypocotyls, dry seeds were surface-sterilised by 2.5% sodium hydrochloride for 30 min, rinsed three times with sterile distilled water and immediately plated in sterile coupled Magenta boxes (77 77 97 mm, Sigma, France) on agar-solidified mineral medium (KNO_3 5 mM, KH_2PO_4 2.5 mM, $MgSO_4$ 0.2 mM, $Ca(NO_3)_2$ 0.2 mM, H_3BO_3 70 "M, $MnCl_2$ 14 "M, $CuSO_4$ 0.5 "M, $NaMoO_4$ 0.2 "M, NaCl 10 "M, $ZnSO_4$ 1 "M, $CoCl_2$, 0.01 "M) containing vitamins (myo-inositol 555 "M, thiamine 3 "M, pyridoxine 5 "M, nicotinamide 8 "M, pantho- thenic acid 2 "M, biotin 0.04 "M) and ferric ammonium citrate (0.05% w/v). Imbibition was performed for 48 h at 4 C, and the culture boxes were then placed in growth chambers (22 C ± 1, 60% ± 10 relative humidity) in the dark or under different light conditions. In the experiments per- formed under far-red light, which inhibits seed germination in tomato, the seeds were first placed in the dark and transferred under far-red light as soon as the radicle protruded from the seed coat.

Light sources

The white light is obtained by filtering light from fluorescent tubes (Prestilux 36 W, Mazda) through neutral filters (heavy frost n129 and neutral den- sity n210, Lee Filters) to obtain a 5 "E m^2 s^1 photon flux. The red light (3 "E m^2 s^1) is obtained by filtering light from fluorescent tubes (TLD red 36W, Philips) through one layer of red polyester filter (primary red n106, Lee Filters). The blue light (10 "E m^2 s^1) is obtained by filtering light from blue fluorescent tube (TLD blue 36W, Philips) through one layer of blue polye- ster filter (special medium blue n363, Lee Filters) and one layer of neutral filter (neutral density n210, Lee Filters). The far-red light is obtained by filtering light from fluorescent tubes (far-red tubes, Sylvania) through red and blue polyester filters (primary red n 106 and dark blue n 119, Lee Filters). Finally, the green safe light is obtained by filtering light from fluorescent tubes (TLD green 36W, Philips) through four layers of green polyester filter (primary green n139, Lee Filters).

Northern-blot analysis and quantification

Seedlings (100–200 for each sample) grown for 7 days were individually harvested. The hypo- cotyls were excised and immediately frozen in liquid nitrogen. Total RNA was extracted using a CsCl cousin method as described by Leprince et al. (1998). Five to 10 "g of each RNA sample were separated on a 1.2% (w/v) agarose formaldehyde/ Mops gel, transferred to a nylon membrane (Biodyne plus, PALL) and UV fixed. Prehybridi- sation and hybridisation of filters were performed at 65 C as described by Church and Gilbert (1984). Probes corresponding to the tomato LeEXT gene (Catala et al. 1997), to the Arabidopsis actin gene (Kerckhoffs et al. 1996) or to the Arabidopsis 18S RNA were labelled with ^{32}P dCTP by random priming (Ready To Go Kit, Pharmacia). Filters were washed at 65 C, once for 5 min and twice for 15 min with 2 SET (NaCl 300 mM, EDTA 4 mM, Tris–HCl 60 mM, pH 7.4), and twice for 15 min with 2 SET, 0.1% SDS. The hybridised filters were then exposed for 4–24 h and the signals were quantified (Storm, Molecular Dynamics). The actin and the 18S probes were used to standardise the amount of RNA loaded, after boiling the CEL7-hybridised membranes in 0.1% SDS for 1 min. The results presented correspond to the standardised quantitation of LeEXT transcripts, expressed as the percentage of the indicated control.

Results

Blue light represses expression of the LeEXT gene via cryptochrome 1 (cry1) photoreceptor

In order to analyse the interaction between light and auxin transduction pathways, we looked for genes regulated by both factors and chose LeEXT, whose expression is strongly up-regulated in tomato hypocotyls after 12–24 h of auxin treat- ment (Catala et al. 1997). The light regulation of the steady state level of LeEXT mRNAs was ana- lysed in 6 day-old wild-type seedlings after 24 h of various light treatments: white (WL), red (R), farred (FR) and blue (B). As shown in Figure 1, we observe, in these conditions, a weak down- regulation of the LeEXT transcript level by white light, and a dramatic inhibition of LeEXT expres- sion after a blue light treatment. We did not observe any clear involvement of red nor far-red lights.

In order to precise the role of the different light qualities in the control of the steady state level of LeEXT mRNAs, and to identify the photorecep- tors involved in light perception in each case, LeEXT expression was analysed in different photoreceptor mutants including fri, tri, phyB2, aurea, cry1 and the multiple mutant fri-tri-phyB2 (Figure 2).

We did not observe any effect of red lights (red: Figure 2A; far-red: Figure 2B) in the control of LeEXT mRNAs levels in wild type hypocotyls (genotype MM). Moreover, no significant effect of these lights was observed in mutants hypocotyls either. In some cases, photomorphogenic mutants exhibit a lower accumulation of LeEXT mRNAs, but the lack of light effect is similar to the WT. These results exclude the involvement of red lights in LeEXT regulation. In blue light (Figure 2C), a decrease of more than 60% in LeEXT mRNAs levels was observed in the WT and phytochrome simple mutants fri, tri and phyB2. However, this decrease is weaker in the chromophore aurea mutant than in the WT and is not observed in the triple mutant deficient in phyA, phyB1 and phyB2. These data support an involvement of phyto- chromes in the blue light response. Furthermore, the cry1 mutant totally lacks the blue light inhibi- tion of LeEXT expression, demonstrating the major role of the cry1 photoreceptor in this response.

Blue light inhibition of LeEXT expression in cry1 is correlated to inhibition of hypocotyl elongation

As we observed a specific inhibition of LeEXT expression by blue light, dramatically reduced in the cry1-deficient mutant, we also analysed the blue-related inhibition of hypocotyl elongation in this mutant. Figure 3 shows the growth kinetics of the cry1 mutant and the corresponding WT grown under different light conditions. No significant difference was observed between both genotypes in the dark, under red or far-red lights. In contrast, cry1 exhibited a weaker inhibition of hypocotyl growth by blue light, compared to the WT. Cry1 therefore appears to be involved in the control of hypocotyl elongation and LeEXT gene expression.

The dgt mutation does not alter the light regulation of LeEXT

In order to determine the relationships between the auxin- and light-control of LeEXT expression, we first analysed the photoregulation of transcript levels in the auxin-insensitive dgt mutant and in the corresponding WT genotype AC. The dgt mutant does not exhibit any induction of LeEXT expression in response to auxin (Catala et al. 1997) and thus appeared to be a useful model to test the role of auxin in the regulation of this gene by light.

Figure 4 shows that the AC-genotype exhibits a significant blue light-dependent inhibition of LeEXT expression. A red light treatment was done as a control to check if an auxin-related reg- ulation of LeEXT expression could be associated to a skotomorphogenesis (darkness)/photomorpho- genesis (light irradiation) transition or to a specific blue light response. As previously shown for the MM genotype, red light has no effect on LeEXT expression. The dgt mutation does not alter the mRNAs steady state in darkness. The similarity of AC and dgt dark controls allows us to consider that the light-induced decrease of the transcripts level observed after the blue irradiation in both genotypes is strictly light dependent. The compar- ison between the dgt mutant and its corresponding WT pointed out an identical regulation of LeEXT mRNAs levels by light. Thus, the insensitivity to auxin conferred by the dgt mutation does not mod- ify the light regulation of LeEXT expression.

Polar auxin transport is required in the blue light regulation of LeEXT expression

In order to check the independence of auxin and blue light transduction pathways in the regulation of the steady state level of LeEXT mRNAs, we studied the role of auxin transport in this photo- regulation. Thus, we

analysed the effect of the auxin transport inhibitor NPA on the LeEXT blue photoregulation in seedlings. As previously, control seedlings switched to photomorphogenesis

were obtained using a red light irradiation. Figure 5 shows that NPA treatments lead to a decrease in LeEXT mRNAs accumulation in darkness or after a red light treatment. These results show that NPA has an inhibitory effect on LeEXT mRNAs accu- mulation, which is consistent with the auxin up- regulation previously described (Catala et al. 1997). As previously shown, blue light inhibits LeEXT expression (see autoradiography, Figure 5), but this inhibition is partially reversed by NPA in a dose-dependent manner. The independence of auxin up-regulation and blue light down- regulation pathways would lead to an additive effect of NPA and blue light on LeEXT expression, or a null effect if we consider that LeEXT expression is almost totally inhibited by blue light. According to the opposite effects of blue light and NPA we observed, we conclude that polar auxin transport is required for the inhibition by blue light of the LeEXT gene.

Discussion

We have chosen to address the question of the role of auxin in the responses of tomato plants to light by studying a simple molecular response in hypocotyls, i.e., the control of the expression of a gene regulated by both factors. We focused on the LeEXT gene, encoding a xyloglucan endo transglycosylase, first, because LeEXT was shown to be strongly up- regulated by auxin (Catala et al. 1997) and second, because the LeEXT protein is putatively involved in the elongation process. Using photoreceptor mutants, we have analysed in detail the photoregu- lation of the steady state level of LeEXT mRNAs. The white light inhibition of LeEXT mRNAs level we observed was expected as light and auxin act antagonistically in the regulation of hypocotyl elon- gation. However, we did not detect any effect of red and far-red lights in this regulation. The high irra- diance red and far-red treatments, as the ones we used, are described as perceived respectively by B-type phytochromes (i.e., phyB1 and phyB2 in tomato) and phyA phytochrome (Fankhauser 2001; Quail 2002). Our results hence suggest the independence of this light response upon the A and B-type phytochromes.

In all the experiments we performed, we observed a strong inhibition of LeEXT transcript levels by blue light. This blue light specificity suggests that the inhibitory effect on the hypocotyl elongation shared by all light signals could be, at least partially, the resultant of regulations of specific targets. This hypothesis is supported by the identification of photomorphogenic mutants specifically impaired in the transduction of a single light signal (Quail 2002). In our work, the cry1 mutant totally lacks the blue light inhibition of LeEXT expression, point- ing out the major role of cry1 in this response. These data can be correlated to the growth kinetics of this mutant showing a defect of the photoregulation of hypocotyl elongation restricted to blue light. LeEXT regulation could be one of the aspects, impaired in cry1, involved in the blue light-related inhibition of hypocotyl elongation.

Furthermore, we did not observe any lack of LeEXT expression regulation in the phytochrome simple mutants. A decrease of this response is sig- nificant in the chromophore mutant aurea, thought deficient for the functionality of all phytochromes, as in the phyA, phyB1, phyB2 triple mutant. These data highlight the involvement of phytochromes in the blue light-related inhibition of LeEXT expres- sion. Because this role could be revealed only in the case of several non-functional phytochromes, each of them should interact with the cry1 transduction pathway in a redundant manner. Such interactions between photoreceptors were previously described at the molecular level in Arabidopsis (Ahmad et al. 1998) and in the physiological regulation of tomato hypocotyl length by blue light (Weller et al. 2001). In this latter work, carried out in similar conditions as those we used, the authors demonstrated com- parable overlapping functions of the different phy-tochromes in the regulation of elongation by blue light, as we described for LeEXT regulation.

To our knowledge, this is one of the first works identifying a gene antagonistically regulated by blue light and auxin, pointing out an interaction of both signals at the molecular level. However, in the moss Physcomitrella patens, Imaizumi et al. (2002) described the antagonistic regulations of many developmental processes, including gene expression, by auxin and cryptochrome signals. We obtained contrasting results using the auxin transport inhibitor NPA and the auxin insensitive dgt mutant on blue-light inhibition of LeEXT expression. Both approaches were expected to decrease the auxin efficiency in the LeEXT regula- tion. However, Catala et al. (1997) did not describe any difference between the dgt mutant and the WT in the absence of exogenously supplied auxin. We did the same observation, whatever the light con- dition used. These results could indicate

that the role of the DGT protein in the regulation of LeEXT expression would be very minor in vivo, and could only be revealed when applying high concentrations of auxin.

Under blue light, NPA has no additional effect on the cry1-related inhibition of LeEXT expression, in contrast to the more than 50% inhibition observed in darkness (Figure 5). This has been already described about the control of hypocotyl elongation in tomato (Kraepiel et al. 2001), but cannot be related to the photomorphogenesis versus skotomorphogenesis developmental programs, since NPA inhibits LeEXT expression in de- etiolated seedlings grown under red light (Figure 5). The fact that NPA partially reverts the blue light inhibition of LeEXT expression demonstrates that auxin transport is necessary to the efficient regulation of LeEXT expression by blue light.

Three hypotheses can be proposed to explain the interaction between cry1 and auxin pathways in LeEXT regulation: (i) as previously proposed by Shinkle et al. (1998) for red light and strongly supported by the molecular studies of the calos- sin-like protein BIG in the polar auxin-transport and in some light responses (Gil et al. 2001), blue light could control auxin-transport from its site of synthesis to its hypocotyl target, leading to the regulation of LeEXT expression. As recently demonstrated in the shade avoidance response controlled by phy B-type phytochromes (Tanaka et al. 2002), this hypothesis assigns the role of transmitter of the blue light signal within the plant to auxin and could explain the co-ordinated development of organs in response to blue light. In this case, in blue light condition, NPA would be ineffective in controlling auxin transport and consequently LeEXT expression since this transport would be already inhibited. (ii) One can imagine a photocontrol of auxin metabolism in seedling apex. Blue light would regulate the size of the auxin pool, which would then migrate to the hypo- cotyl and modulate the expression of auxin- regulated genes. (iii) Similarly to the direct phosphorylation of AUX/IAA proteins by phyto- chromes (Colon-Carmona 2000), one can also ima- gine a regulation by blue light, at the target site, of elements of the auxin transduction pathway. Such an hypothesis has been proposed to explain the link, revealed in the dfl1 mutant, between auxin and different light signals, mainly the blue one (Nakasawa et al. 2001). It is also the model proposed by Imaizumi et al. (2002) to explain the disruption of auxin responses in the Physcomitrella cryptochrome mutants. In this model, the decrease in the auxin signal by NPA would lead to the decrease in the effectiveness of the blue light inhi- bitory effect. This third hypothesis supposes the existence of another mechanism different from auxin transport to integrate at the whole plant level the light signal perceived by the different parts of the plant.

The blue light effect on IAA metabolism and transport remains to be elucidated to better under- stand the physiological interactions between blue light and auxin involved in LeEXT regulation and, at the whole plant level, in the control of elongation.

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Note: as we cannot further carry on with this research program, segregations and corresponding T1 tobacco seeds carrying the LeEXT gene in sense/antisense orientations are available from Yvan Kraepiel.

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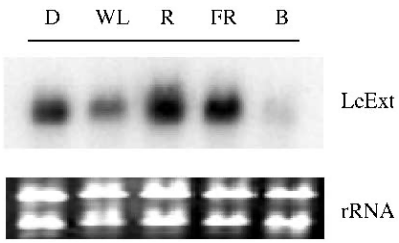

Figure 1. Effect of light quality on LeEXT transcripts accumulation in whole hypocotyls of tomato seedlings. Seedlings (var. MM) were cultivated for 6 days after germination under darkness then transferred to various light conditions (D: darkness, WL: white light, R: red, FR: far-red, B: blue) for 24 h. Total RNA was isolated from hypocotyls. Ten microgram were separated per lane and hybridised with LeEXT probe. The lower panel represents an ethidium bromide gel and visualises the ribosomal RNAs as a control for equal loading and intactness of the RNA.

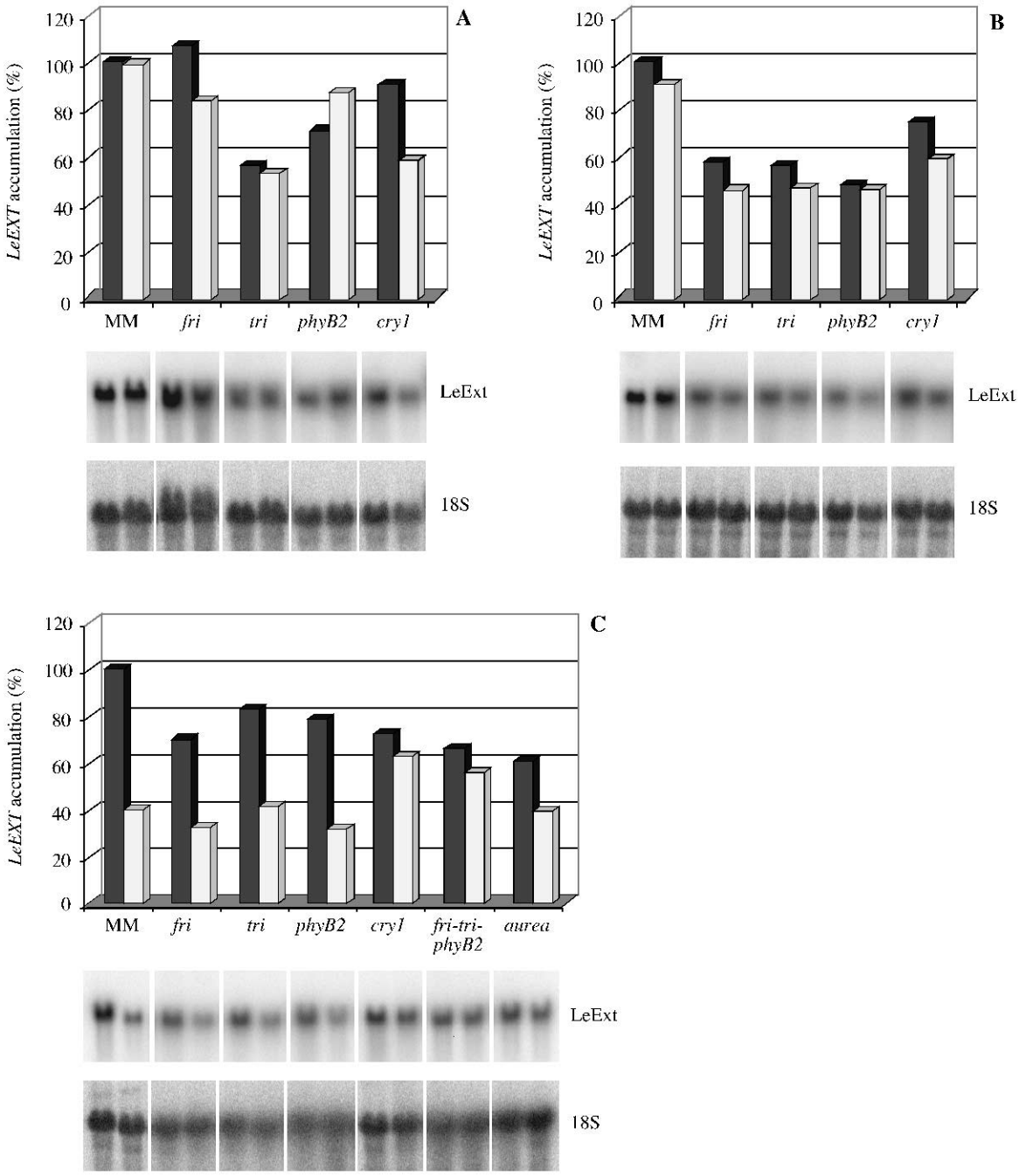

Figure 2. Effect of light quality and photoreceptor mutations on LeEXT transcripts accumulation in whole hypocotyls of tomato seedlings under different light conditions. Wild type (MM), phytochrome mutants (fri, tri, phyB2, aurea, fri-tri-phyB2) and cryptochrome mutant (cry1) seedlings were cultivated for 6 days after germination in darkness then transferred to various light conditions for 24 h. A: red light, B: far-red light and C: blue light. Ten microgram of total hypocotyl RNA were loaded in each lane and hybridised with the LeEXT probe (upper panel) then the 18S rDNA probe (lower panel). LeEXT accumulation in the dark (dark bars) or in the light conditions (light bars) was quantified using the MM dark-grown seedlings as a relative reference. Quantitation was normalised according to the hybridisation of the 18S rDNA probe for RNA loading and intactness. These data are representative of 4–5 repeats done in each case.

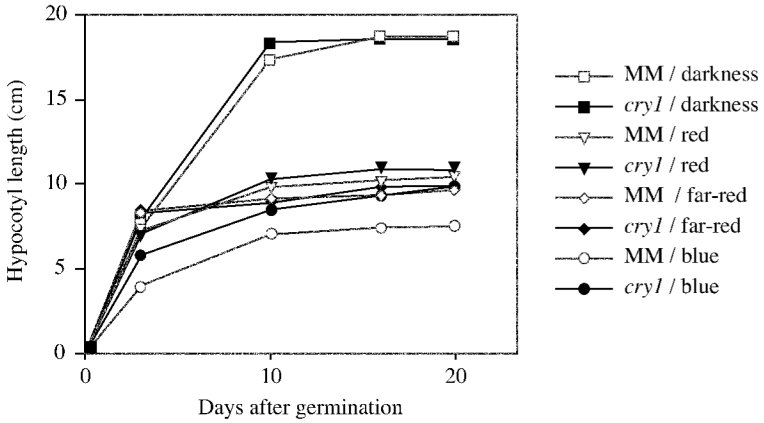

Figure3. Effect of light quality and photoreceptor mutations on hypocotyl elongation kinetics of tomato seedlings. Hypocotyl length of wild type (MM) and cryptochrome mutant (cry1) was measured from day 3 to day 20 in light- and dark-grown seedlings. Each value is a mean of 30–50 measurements ± S.E. (S.E. are included into the points).

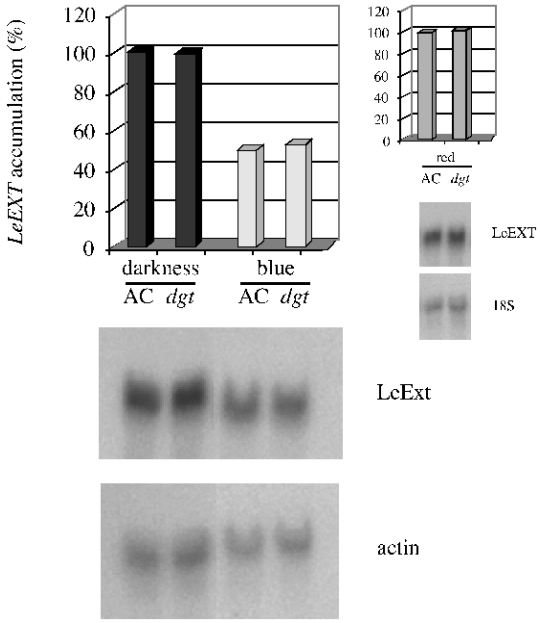

Figure 4. Effect of auxin-insensitive mutation (dgt) on LeEXT transcripts accumulation under darkness and blue light conditions in whole hypocotyls of tomato seedlings. Wild type (AC) and dgt seedlings were cultivated in the same conditions as in Figure 2. RNA extraction, separation and hybridisation were performed as in Figure 2. Quantitation of LeEXT accumulation was done using dark-grown AC seedlings as a relative reference. The red light condition is provided as an irradiated seedlings control. Normalisation was done with the actin probe. These data are representative of three repeats.

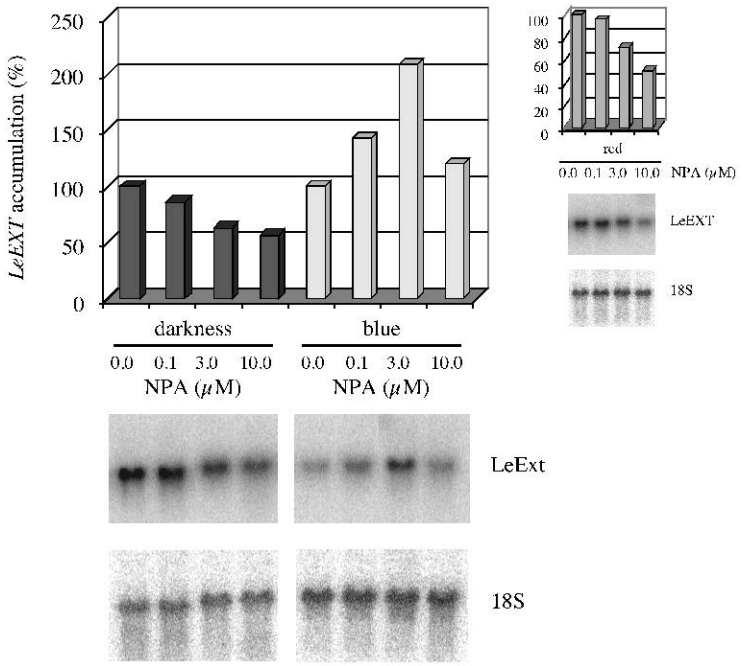

Figure 5. Effect of NPA on LeEXT transcripts accumulation under darkness and blue light conditions in whole hypocotyls of tomato seedlings. MM seedlings were cultivated in the same conditions as in Figure 2, but NPA treatments were applied 3 h before the start of the irradiation. RNA extraction, separation and hybridisation were performed as in Figure 2. Quantitation of LeEXT accumulation was carried out using seedlings without NPA treatment (0.0 "M) as relative reference. The effect of NPA under red light condition is provided as an irradiated seedlings control. Normalisation with the 18S rDNA probe was done as in Figure 2. These data are representative of two repeats.