Regulation of tomato lateral root development by carbon monoxide and involvement in auxin and nitric oxide

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Abstract
Carbon monoxide (CO) is an endogenous gaseous molecule in organisms. Despite its reputation as a lethal gas, recent studies have shown that it is one of the most essential cellular components regulating a variety of biological processes. However, whether CO regulates physiological processes of morphological or developmental patterns in plants is largely unknown. In this paper, the observation that exogenous CO was able to promote the formation of tomato lateral roots (LR) is described. The CO stimulation of LR development was supported by analysis of tomato haem oxygenase-1 (LeHO-1), an enzymatic source of intracellular CO. It is shown that the amount of LeHO-1 proteins and transcripts increased parallel to the LR development. In addition, LeHO-1 loss-of-function tomato mutant yg-2 showed a phenotype of impaired LR development. The phenotype of yg-2 could be restored by treatment with CO. Since auxin is required for LR initiation and NO is shown to be a mediator for LR development, the correlation of CO with auxin and NO was tested. Our analysis revealed that the action of CO was blocked by the auxin transport inhibitor N-1-naphthylphthalamic acid and the NO scavenger cPTIO, respectively. Furthermore, the whole seedling assays of IAA show that treatment with CO increased the overall IAA levels in various tissues of tomato. Exposure of tomato roots to CO also enhanced intracellular NO generation. These results indicate that CO plays a critical role in controlling architectural change in tomato roots.

Key words: Carbon monoxide, haem oxygenase-1, IAA, lateral root, NO.

Introduction
Root branching is a major determinant of plant architecture, which plays a principal role in water-use efficiency and the extraction of nutrients from soils. In Arabidopsis, lateral roots (LRs) are found to be derived from lateral root primordia, and the latter, in turn, is formed from a subset of pericycle cells, termed pericycle founder cells adjacent to the two xylem poles (Malamy and Benfey, 1997; Casimiro et al., 2003). Growing evidence has shown that auxin is a key factor controlling LR initiation (Casimiro et al., 2003). Overgeneration of auxin or the application of exogenous auxin improves the number of lateral roots, whereas blocking polar auxin transport at the interface of the root inhibits lateral root initiation (Blakely et al., 1988; Laskowski et al., 1995; Casimiro et al., 2001; Malamy, 2005). For example, plant roots deprived of endogenous auxin by growing them in the presence of the auxin transport inhibitor N-1-naphthylphthalamic acid (NPA) fail to form lateral roots (Casimiro et al., 2001). Genetic studies also indicate that the formation of LR primordia involves dynamic gradients of auxin with maxima at the primordial tips, and these gradients are mediated by a cellular efflux requiring asymmetrically localized PIN proteins (Benková et al., 2003). In addition, the GNOM gene coding an ARF GDP/GTP exchange factor for the co-ordinated polar localization of PIN proteins (Geldner et al., 2004) and the auxin influx carrier AUX1 (Marchant et al., 2002), as well as the auxin-regulated AP2/EREBP
gene PUCHI, also mediate lateral root initiation (Hirota et al., 2007).

Although lateral root development is controlled by auxin and other important components in the auxin signal transduction pathway (Reed et al., 1998; Himanen et al., 2002; Benkóva et al., 2003; Zhu et al., 2006), the placement and formation of lateral root are strongly influenced by physiological state and the prevailing environmental conditions. Nutrients, such as NO\(_3^-\) or Pi, can be one of the major environmental signals that affect LR development (Robinson, 1994; Forde and Lorenzo, 2001). Nitric oxide (NO) has been reported to affect LR initiation (Correa-Aragunde et al., 2004). The application of exogenous NO increased the number of tomato lateral roots, whereas depletion of intracellular NO arrested LR initiation.

Like NO, carbon monoxide (CO) is endogenously produced in a variety of organisms in micromolar quantities (Hartsfield, 2002). For example, carbon monoxide is formed during the growth of cucumber seedlings in the dark in the atmosphere or in the germinating seeds of rye, cucumber, and other species (Wilks, 1959; Siegel et al., 1962). Recently, CO has been highly appreciated for its versatile properties as a signalling mediator and an active regulator of physiological processes in animals (Boczkowski et al., 2006). Several reports have shown that both endogenously generated CO and low doses of applied CO gas exert beneficial effects, such as anti-inflammatory, anti-apoptotic, and cytoprotective actions (Otterbein, 2002). In plants, CO has been reported to regulate the germination of seeds (Siegel et al., 1962). CO also mediates the response of bacterium Desulfovibrio desulfuricans to oxidative stress (Davydova and Tarasova, 2005).

Intracellular generation of CO and its action are closely linked to the haem oxygenase (HO, EC 1.14.99.3) enzyme present in both animals (Kikuchi et al., 2005) and plants (Davis et al., 1999; Muramoto et al., 1999). Haem oxygenase catalyses the degradation of haem to produce carbon monoxide, free iron, and biliverdin. Of these metabolites, biliverdin is converted to bilirubin by bilirubin reductase, and bilirubin itself acts as a direct antioxidant (Noriega et al., 2004). Carbon monoxide has several biological functions including the regulation of reactive oxygen species (ROS) generation and signalling of the oxidative stress response in cells (Boczkowski et al., 2006; Srisook et al., 2006). While animal haem oxygenases are involved in a catabolic process of haemoglobin in senescent red blood cells with the concomitant recycling of iron (Richaud and Zabulon, 1997; Kikuchi et al., 2005), the plant haem oxygenases are involved in root development (Cao et al., 2007) and the synthesis of tetrapyrrole chromophores, phycobilins, and phytochromobilins, thus regulating photomorphogenesis (Muramoto et al., 1999, 2002; Davis et al., 2001).

Genes of haem oxygenase families have been separated and identified in various organisms (Kikuchi et al., 2005). HO-1 is an inducible form that is transcriptionally up-regulated by a variety of chemical and physiological stress-inducing factors, for example, heavy metals (Srisook et al., 2005), hydrogen peroxide (Keyse and Tyrrell, 1989), and heat shock (Shibahara et al., 1987). In addition, HO-derived CO is assumed to function, like nitric oxide (NO), as a physiological regulator of cGMP by activating guanylyl cyclase (Baranano and Snyder, 2001). Although several reports have demonstrated that plant HO regulates phytochrome chromophore biosynthesis (Davis et al., 1999, 2001; Muramoto et al., 1999), additional physiological functions of this enzyme are largely unknown. In this study, the regulatory role played by CO and haem oxygenase-1 in tomato lateral root development has been investigated. Evidence is provided that CO-induced LR initiation is closely correlated with the auxin-responsive and NO-mediated LR development pathway. This work may improve our understanding of regulatory functions of CO and HO-1 for plant lateral root development.

Materials and methods

Plant materials and growth conditions

Seeds of tomato (Solanum lycopersicum, lines KK and yg-2 in the KK background, as well as SH-2003) were soaked in distilled water and germinated on a mesh tray floating on 1.0 litre of solution containing 0.1 mM CaSO\(_4\) (pH 5.6). After germination, seedlings were transferred to the quarter-strength Hoagland’s nutrient solution and grown at 24±1°C, 100 μmol m\(^{-2}\) s\(^{-1}\) light intensity, and a 12 h photoperiod for 48 h. When the average root length was about 6–8 mm, CO was supplied to seedlings by adding CO-saturated water to the root-bathing solution. The seedlings were treated with CO at different concentrations once a day. Treatment solutions were changed daily.

CO determination

The pure CO gas was provided by the Institute of Special Gas in Beijing, China. Bubbled CO gas gently went through a 3 mm (i.d.) glass tube into 250–500 ml of water in an open tube for 15–20 min. The CO in the water was quantified spectrophotometrically by the formation of carboxyhaemoglobin (HbCO) described by Chalmers (1991). The principle of the method is to allow the CO in the water to interact with a solution of haemoglobin and then to measure HbCO formed by using the dithionite reduction at 541 nm and 555 nm. The amount of pure CO in water solution was calculated and expressed as μmol l\(^{-1}\).

IAA measurement

Harvested seedlings were rinsed with distilled water, blotted dry, and immediately weighed. The tissue samples were homogenized in a solution of methanol. The extract was passed through a C\(_{18}\) column on a solid phase extractor and the eluate collected. Then 1 ml methanol was continuously added to the C\(_{18}\) column. IAA in the extract was quantified by HPLC (Waters 515, Waters Technologies Co. Ltd.) with an ultraviolet (UV) detector (254 nm) and a quantitative tube that automatically and accurately controls the
set volume injected. The operating conditions were a Hypersil reversed phase C18 column (μ Bondapak, 250 mm×4.6 mm i.d.), a mobile phase of methanol/water (4:1; v/v), a flow rate of 0.6 ml min⁻¹, an injection volume of 20 μl, and a temperature of 25 °C. Under these conditions, the retention time for IAA was 3.9 min.

**Immunohistochemical detection of LeHO-1 protein**

Peptide sequences containing specific amino acids corresponding to the LeHO-1 sequence positions for the antigen were obtained by chemical synthesis. The sequence was hydrophilic, surface-oriented, and flexible. Synthetic peptide was purified by HPLC and coupled to keyhole limpet haemocyanin (KLH). The LeHO-1-KLH was collected and used for producing the anti-LeHO-1 antibody. For the LeHO-1 protein assay, harvested roots were frozen in liquid nitrogen, homogenized and extracted with a buffer solution containing 0.1 M TRIS/HCl (pH 7.8), 1 mM EDTA, 1 mM phenylmethylsulphonyl fluoride, 0.7 mg ml⁻¹ pepstatin, 1 mg ml⁻¹ aprotinin, 0.5 mg ml⁻¹ leupeptin, and 2 mM DTT. Extracted proteins (30 mg) were separated by electrophoresis on a 12% SDS-polyacrylamide gel and blotted onto polyvinylidene difluoride membranes. Protein gel blot analysis was performed using the anti-LeHO-1 antibody as the primary antibody and horseradish peroxidase-conjugated anti-rabbit IgG as the secondary antibody according to the method described previously by Muramoto et al. (1999).

**Detection of intracellular NO**

Detection of root cellular NO was performed by the method described by Correa-Aragunde et al. (2004). Seedling roots were exposed to various concentrations of CO and then transferred to 20 mM HEPES-NaOH (pH 7.5) buffer solution containing 15 μM specific NO fluorescent probe 4,5-diaminofluorescein diacetate (DAF-2DA). After incubating in darkness for 15 min, the roots were washed several times and immediately visualized (excitation 490 nm and emission 525 nm) using a fluorescence microscope (Axio Imager, A1, Zeiss).

**RT-PCR analysis**

Total RNA was extracted from root tissues using Trizol (Invitrogen, Carlsbad, CA). Reverse transcription was carried out at 37°C for 1 h with M-MLV reverse transcriptase (Promega, Madison, WI). The first strand cDNA was then used as a template for polymerase chain amplification and to analyse the amount of transcripts of tomato LeHO-1. The amplification primers for LeHO-1 were: 5'-CAACAACCTGCGCTGAGAAATC-3' (sense) and 5'-AAAATCCGCGTCCTCCAATTTG-3' (antisense) (accession number AF320028). Expression of Actin gene (sense 5'-AGAACCTATAGACCTCCAGATGG-3' and antisense 5'-TTAATCTTCACTGTGCTAGGAC-3') was used as a control. The optimized linear range for LeHO-1 and Actin amplification was determined as 23 cycles. The PCR reaction conditions were 30 s at 94°C, 30 s at 56°C, and 40 s at 72°C for 23 cycles. Each PCR experiment was performed three times with different cDNA sets from independent biological replicates. The PCR products were applied to 1% (w/v) agarose gel electrophoresis and stained with ethidium bromide.

**Statistical analysis**

Each result shown in the figures was the mean of three replicated treatments, and each treatment contained at least 15 seedlings. The significant differences between treatments were statistically evaluated by standard deviation and Student’s t test methods.

**Results**

**CO induces lateral root formation**

To investigate the role of CO in regulating tomato lateral root development, 2-d-old seedlings were exposed to various concentrations of CO. As shown in Fig. 1, the number of lateral roots increased in response to CO exposure of 1 μM to 200 μM. The maximal number of LR was observed at 10 μM of CO, where an 8.2-fold increase in LR number was achieved relative to the control. By contrast, the primary root (PR) length decreased proportionally with increasing CO concentrations (Fig. 1c). To examine whether CO regulation of LR development is a general mechanism, both Arabidopsis and Brassica napus were tested. It is shown that these plant species displayed a similar morphological response (Fig. 1d, e; see Supplementary Table S1 at JXB online). Rapeseed seedlings have been shown to develop more lateral roots when exposed to exogenous CO (Cao et al., 2007). The formation of LR primordia in CO-exposed roots was also determined. Treatment with 10 μM CO for 36 h induced marked increases in LR primordia relative to the control (Fig. 2a). This induction was evident for 48 h. On the other hand, the emergence of LR with CO occurred at 48 h after the experiments began, and the number of lateral roots then increased rapidly (Fig. 2b). The development of lateral roots in the control seedlings was relatively slow, with more than a 24 h lag behind that of CO-treated roots.

Since the CO molecule is a strong poisonous gas and can block mitochondrial oxidative phosphorylation by binding to respiratory cytochromes (Singh, 2002; Srisook et al., 2006), the activity of cytochrome c oxidase was therefore determined in plants exposed to CO. The analysis of cytochrome c oxidase showed that activity of the enzyme was not affected by CO at 0.1–50 μM (data not shown).

**Formation of lateral roots is regulated by HO-1**

Several lines of study have demonstrated that haem oxygenase-1 (HO-1) regulates the biosynthesis of phytchrome chromophores by cleavage of haem (Muramoto et al., 1999; Davis et al., 2001). HO-1 loss-of-function mutants, such as the Arabidopsis long hypocotyl (hy)-1 (Parks and Quail, 1991), pea phychromophore-deficient (pcd)-1 (Weller et al., 1996), tomato yellow-green (yg)-2 (Terry and Kendrick, 1996), and rice photoperiodic sensitive (se)-5 (Izawa et al., 2000), show multiple defective phenotypes including a yellow-green colour (Davis et al., 2001) and a long hypocotyl (Oyama et al., 1997; Muramoto et al., 1999). Among the phenotypes, one defect that seems to be missing is the delayed development of lateral roots. Since CO can be generated by HO-1 and HO-1 is considered as an intracellular source of CO (Verma et al., 1993), it is hypothesized that HO-1-derived CO would regulate lateral root development.
in the same way as external carbon monoxide. To test this possibility, the tomato mutant *yg-2* was used for identifying its function. As shown in Fig. 3a, the *yg-2* mutants produced few or no lateral roots. Also, its counterpart, the *hy1* mutant from *Arabidopsis* was phenotypically similar, showing no lateral roots (Fig. 3b). Interestingly, when the *yg-2* and *hy1* seedlings were fed exogenous CO, the phenotype of the lateral roots was restored (see Fig. 1).
Supplementary Table S2 at JXB online). These results suggest that HO-1 is most likely to participate in the regulation of LR development.

To relate HO-1 to LR development, a quantitative RT-PCR-based assay was performed to analyse the transcript amounts of LeHO-1. At the early stages of LR development, the expression of LeHO-1 was very low in the roots (Fig. 4a). The control roots began to accumulate LeHO-1 transcripts at 72 h. The roots exposed to CO showed a similar pattern to LeHO-1 expression, indicating that treatment with CO did not affect LeHO-1 expression at the transcription level. To support the fact that the enhanced formation of LR was associated with the expression of LeHO-1, protein gel blot analysis of LeHO-1 was performed in tomato roots, using LeHO-1-specific polyclonal antibodies. Expression of LeHO-1 protein in the control roots was not induced during the initial 72 h, but following that time, a band of 26 kDa was detected (Fig. 4b; see Supplementary Fig. S1 at JXB online). The molecular mass of LeHO-1 is similar to that of Arabidopsis HO-1 (Muramoto et al., 1999). The pattern of LeHO-1 expression in tomato roots was well matched with their LR emergence under normal conditions. In CO-treated roots, the LeHO-1 protein was detected at 72 h after the experiment began, showing a parallel change with LR emergence. Collectively, these studies indicate that either exogenous or endogenous CO was able to induce the formation of lateral roots in tomato.

**CO-promoted lateral root formation is involved in auxin and NO action**

It is well known that lateral roots are initiated from the subepidermal layer, the pericycle, and only the pericycle

Fig. 3. CO-induced emergence of lateral roots in tomato mutant yg-2 (a) and Arabidopsis mutant hy1 (Landsberg background) (b). Two-day-old tomato seedlings [(WT: wild type (KK); MT, Mutant: yg-2, (LA2469A)] after germination were treated 10 μM CO for 4 d. For Arabidopsis, seedlings were cultured in half-strength MS solid medium for 4 d after germination and then the medium was supplemented with CO at 0 and 10 μM for 6 d. After that, the roots were photographed under a light microscope (bar = 1 cm).

Fig. 4. The amount of transcripts and proteins of tomato root LeHO-1 during the development of lateral roots exposed to CO. Tomato seedlings were grown hydroponically for 2 d after germination and then treated with 10 μM CO for 0, 24, 48, 72, and 96 h. (a) Analysis of LeHO-1 transcripts by semi-quantitative RT-PCR. Actin was used for cDNA normalization. The number below the band indicates relative abundance (RA) of HO-1 with respect to the loading control actin. (b) Immunoblot analysis. Extracts from tomato roots were analysed by protein gel blotting using an antibody raised against LeHO-1 proteins. The molecular mass of the proteins is indicated on the right in kilodaltons. The results shown above were from one of the three independent experiments.
cells adjacent to the pretoxylem poles have the capacity to generate lateral roots (Xie et al., 2000). Auxin stimulates LR formation by activating pericycle cell division (Himanen et al., 2002; Woodward and Bartel, 2005; Wu et al., 2007). To examine whether the CO-promoted LR emergence was involved in the auxin response pathway, 2 d tomato seedlings were incubated with N-(1-naphthyl) phthalamic acid (NPA), an inhibitor of polar auxin transport (Reed et al., 1998; Casimiro et al., 2001). As shown in Fig. 5, NPA at 50 nM could completely abolish CO-promoted LR emergence. This suggests that CO-promoted LR development might be dependent on the auxin-responsive pathway of LR development. To confirm the correlation between CO and auxin, the tomato mutant yg-2 was used to test the effect of auxin on LR formation with a deficiency in intracellular CO. Our analysis demonstrated that the mutants were able to develop lateral roots when treated with 50–100 nM 1-naphthalene acetic acid (Fig. 6a, b), strongly suggesting that CO-induced lateral root formation is closely associated with the auxin signalling pathway. Similarly, the elongation of the primary root and the hypocotyl was shown to be inhibited (Fig. 6c, d). To show the role of CO in regulating auxin-responsive LR development, the IAA in CO-treated seedlings was quantified. Treatment with 10 μM CO induced moderate but significant increases in IAA content in shoots relative to the controls (Fig. 7). However, increasing CO concentrations did not promote IAA accumulation further. CO-induced IAA accumulation was also observed in tomato roots. Although CO at 10 μM induced a slight increase in IAA accumulation, a higher level of CO up to 50 μM induced more IAA accumulation in roots.

Nitric oxide (NO) is a biologically active gaseous molecule and is proposed to be one of the important second messengers in plant cells (Durner and Klessig, 1999). Since NO has also been reported to be required for LR development in tomato (Correa-Aragunde et al., 2004, 2006), we were interested in testing the role of CO in regulating NO generation. A NO specific scavenger, cPTIO, was used to treat tomato seedlings in the presence of CO. It was shown that treatment with 1 μM cPTIO cancelled CO-promoted LR emergence (Fig. 5). To confirm that CO-regulated LR formation was dependent on NO action, the production of NO in tomato roots was detected using DAF-2DA fluorescent emission. Control roots displayed only very light NO intensity around the root apex region, but roots exposed to CO at 10 μM and 50 μM stained extensively (Fig. 8a). The NO accumulation and distribution surrounding the area where primordia typically developed were also examined. As shown in Fig. 8b, NO was only found around the area where primordia initiated. However, no difference of NO staining between the CO treatment and control was observed. Additional experiments were performed to see whether NPA or cPTIO could regulate the expression of LeHO-1. Our results showed that LeHO-1 expression was not regulated by NPA or cPTIO (see Supplementary Fig. S2 online).

**Discussion**

Recent studies have demonstrated that carbon monoxide is produced in trace amounts by various organisms as an essential mediator (Hartsfield, 2002). It is known that CO is physiologically active in animal cells with versatile functions (Prockop and Chichkova, 2007). In animals, there are at least two major aspects of CO regulatory function. One is the typical biological reactivity of CO, which is represented by its interaction with iron-containing proteins. These proteins containing either haem or [Fe–S] clusters are one of the most ubiquitous and functionally versatile groups in organisms (Boczkowski et al., 2006). Another role of CO is that CO functions as a signal molecule in cells by interaction with NO synthase, cytochrome oxidase, mitogen-activated protein kinase (MAPK), and K+ channels, regulating a variety of downstream physiological responses (Boczkowski et al., 2006). However, the physiological processes for CO action in plants are largely unknown. In this study, it is demonstrated that exogenous CO was able to promote the formation of tomato lateral roots. The CO-induced LR formation was showed to be concentration-dependent.
With a low dose of CO (1–10 lM), the seedlings showed an enhanced LR formation, whereas at higher levels (more than 50 lM), it resulted in the inhibition of root growth. This pattern can also be found when using other signal molecules/metabolites such as NO (Correa-Aragunde et al., 2004; Wang and Yang, 2005), auxin (Blakely et al., 1988; Laskowski et al., 1995), brassinosteroids (Bao et al., 2004), and alkamides (Ramírez-Chávez et al., 2004). However, tomato primary root growth was inhibited with the concentrations of CO applied. This was more obvious when the CO concentrations exceeded 50 lM. The possible reason for this is the toxicity of CO at a level in excess of the physiological requirements. It is also true that plant hormones like auxin (e.g. NAA) and nitrogen oxides (NO) at higher levels also inhibit the growth of primary roots (Correa-Aragunde et al., 2004).

The formation of tomato LR promoted by CO could be supported by an analysis of haem oxygenase-1, which is believed to be an enzymatic source of CO (Kikuchi et al., 2005). Both LeHO-1 expression and the amount of LeHO-1 proteins were up-regulated during LR development (Fig. 4). In addition, the LeHO-1 loss of function yg-2 mutant of tomato and the hy1 mutant of Arabidopsis show the same phenotype of impaired lateral root development, suggesting that HO-1 could be responsible for lateral root development. Moreover, the phenotype of the tomato yg-2 mutant can be restored by feeding with exogenous CO, comparable to the Arabidopsis hy1 mutant which, when transformed with the AtHO1 gene, exhibited a wild-type phenotype (Davis et al., 1999).

It is known that auxin polar transport, local accumulation, and redistribution are required for the formation of primordia and lateral roots (Casimiro et al., 2001; Benková et al., 2003; Kramer and Bennett, 2006). Treatment of plant roots with auxin transport inhibitors such as NPA blocks lateral root formation, whereas the application of exogenous auxin (NAA), improves lateral root development (Casimiro et al., 2001; Himanen et al., 2002; Hirota et al., 2007). During the process of LR development, auxin produced in shoots is transported through the stele to the root tips and redistributed to the other layers of root cells (Jones, 1998; Kramer and

**Fig. 6.** Effect of 1-naphthalene acetic acid (NAA) on the LR emergence of tomato mutant yg-2 (LA2469A). Seedlings were grown hydroponically for 2 d after germination and then treated with the indicated concentrations of NAA for 5 d. (a) The number of yg-2 lateral roots (LR) exposed to the indicated concentrations of NAA. (b) Photograph of yg-2 lateral root emergence. (c) Change of elongation of the primary root (PR). (d) Hypocotyl elongation. Values represent the mean of three independent experiments and vertical bars indicate standard deviations (n=45 seedlings). Asterisks indicate that the mean values are significantly different between the NAA treatments and controls (P <0.05). The bar in the graph (b) indicates 1 cm.
A functional interaction of carbon monoxide with auxin was demonstrated in the tomato root. It is shown that CO-promoted formation of lateral roots was suppressed by the auxin transport inhibitor NPA (Fig. 5). Treatment with NAA could restore the root phenotype of yg-2 (Fig. 6). The whole seedling assays of IAA also revealed that CO was able to increase the overall IAA levels in various tissues of tomato seedlings (Fig. 7). These results suggest that CO may cross-talk with auxin or interact with auxin-responsive signal transduction cascades via altering biosynthesis/perception in some way, thus leading to the modification of lateral root development. It is also noted that, in contrast to auxin that induces the formation of primordia and lateral roots, CO seemed only to induce the emergence of lateral roots. The mechanism for the phenomenon is unclear. But the possibility cannot be ruled out that CO may partially cross-talk with auxin-responsive LR development.

Analysis of HO-1 interaction with auxin signalling in lateral root development would also be interesting. Previous studies have shown that the HY1 locus (HO-1) of Arabidopsis is required for phytochrome chromophore biosynthesis (Davis et al., 1999; Muramoto et al., 1999). This process regulates plant morphogenesis including hypocotyl elongation, leaf expansion, and apical dominance. In this case, auxin participates in the phytochrome-mediated light signal, which might be further transduced to the root cells for their responses. Understanding the cross-talk between HO-1 and auxin would be a challenge for further research. In addition, the identification of major genes or proteins, such as NAC1 (Xie et al., 2000), PIN (Reinhardt et al., 2003; Benkova et al., 2003), and PUCHI (Hirota et al., 2007) mediating the auxin signal for root development, would also improve our understanding of the process of CO-mediated LR development.

Nitric oxide plays various physiological roles in both plants and animals. Recently, several studies have indicated that low levels of NO are able to mediate auxin-controlled LR development (Correa-Aragunde et al., 2004, 2006). In this study, the participation of CO in the process of NO-regulated LR development has been demonstrated. Treatment with CO induced intracellular NO generation during LR primordia initiation, suggesting that CO may interact with putative proteins responsible for NO production. In animals, some enzymes including guanylate cyclase and NO synthase have been described as the targets of CO, and these enzymes are shown to be involved in CO signalling (White and Marletta, 1992; Verma et al., 1993). Several other proteins, such as cytochrome oxidase, mitogen-activated protein kinases (MAPKs), and K+ channels, interacting with CO directly,

Fig. 7. CO regulation of IAA accumulation and distribution in tomato root, leaf, and stem during lateral root development. Seedlings were grown hydroponically for 2 d after germination and then treated with the indicated concentrations of CO for 12 h. After that, the tissues were sampled and IAA was measured by HPLC. Values represent the mean of two independent experiments and vertical bars indicate the standard deviations (n=90 seedlings). Asterisks indicate that the mean values are significantly different between the CO treatments and controls (P < 0.05).

Fig. 8. Visualization of in vivo NO generation in tomato roots exposed to carbon monoxide. (a) Visualization of NO in primary roots. Seedlings were treated with CO at 0, 10, and 50 µM for 24 h. After treatments, the seedling roots were loaded with 15 µM 4,5-diaminofluorescence (DAF-2DA) for 15 min and immediately photographed (bar = 5 mm). (b) Visualization of NO in lateral roots. Seedlings were treated with 0 and 10 µM CO for 12, 24, 36, 48, and 60 h and then exposed to DAF-2DA for 15 min. After that, they were immediately photographed (bar = 10 mm).
are also thought to be the important components in the CO signalling pathway (Boczkowski et al., 2006). In plants, however, little is known about the interaction between CO and NO. It is probable that CO may regulate genes that are encoding proteins responsible for NO generation. In addition, CO and HO-1 may modulate redox signalling by interaction with haem and NADPH oxidase, thus affecting homeostasis of reactive oxygen species. Future studies in this direction will help to define the physiological role of low doses of CO in regulating the cellular generation of NO and ROS, and the network within CO, NO, and ROS.

In conclusion, evidence is provided indicating that carbon monoxide was able to regulate tomato lateral root development governed by both auxin and the NO signalling transduction pathway. This process can be supported by the genetic and physiological observations that the formation of lateral roots was closely linked to the tomato CO-generated enzyme LeHO-1. Since auxin is required for lateral root initiation and NO is shown to mediate this process, it is shown that CO was able to mimic to some extent the action of auxin and NO during tomato LR development. These results suggest that CO represents another signal mediator for plant root development. Further research will be required for understanding the interaction between CO and auxin transport and distribution in roots more clearly. Also, more detailed investigations are required to identify the molecular basis of HO-1 in regulating other genes for LR development.

Supplementary data

Supplementary data for this article are available at JXB online.

Supplementary Fig. S1. The amount of tomato root LeHO-1 proteins during the development of lateral roots exposed to CO.

Supplementary Fig. S2. The amount of transcripts of tomato root LeHO-1 during the development of tomato lateral roots exposed to CO, NPA, and cPTIO.

Supplementary Table S1. Effect of CO on the lateral root development of Arabidopsis (ecotypes, Landsberg erecta) and rapeseed (Brassica napus).

Supplementary Table S2. CO-induced emergence of lateral roots in the tomato mutant yg-2 and the Arabidopsis mutant hy1 (Landsberg background).

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