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Sucrose and folates modulate auxin signalling

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Research Category:
System Biology, Molecular Biology, and Gene Regulation
Full title of the paper:

Interplay between sucrose and folate modulates auxin signalling in Arabidopsis.

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Funding:
Research infrastructure and technical support was provided by the Centre for Analysis of Genome Evolution and Function at University of Toronto. This work was generously supported by funding from the Natural Science and Engineering Research Council of Canada (NSERC), the Canadian Foundation for Innovation (CFI), and the University of Toronto to MMC.

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ABSTRACT

As sessile organisms growing in an ever-changing environment, plants must integrate multiple regulatory inputs to promote the appropriate developmental responses. One such nutritional signal is cellular sugar levels, which rise and fall throughout the day and affect a variety of developmental processes. To uncover signalling pathways that modulate sugar perception, compounds from the library of active compounds in Arabidopsis (LATCA) were screened for the ability to perturb developmental responses to sucrose in Arabidopsis thaliana seedlings. This screen found that sulfonamides, which inhibit folate biosynthesis in plants, restrict hypocotyl elongation in a sugar-dependent fashion. Transcriptome analysis identified a small set of transcripts that respond the interaction between sulfonamide and sucrose, including a number of transcripts encoding Aux/IAAs, negative regulators of auxin signal transduction. Chemical inhibition of auxin transport or genetic disruption of auxin signalling relieved this interaction, suggesting that responses to these two nutritional stimuli are mediated by auxin. Reporter systems used to track auxin signalling and distribution showed enhanced activity in the vascular region of the hypocotyl in response to co-treatment of sucrose and sulfonamide, yet no change in auxin abundance was observed. Taken together, these findings suggest that the interplay between sucrose and folates acts to fine-tune auxin sensitivity and influences auxin distribution during seedling development.
INTRODUCTION

Sugars play a multifaceted role in plant biology, acting as structural components, sources of energy, and signalling molecules (Rolland et al., 2006). The effect of sugars on development can be seen across all stages of the plant life cycle, from germination and establishment (Zhou et al., 1998; Rognoni et al., 2007), through vegetative growth and floral transition (Ohto et al., 2001; Corbesier et al., 2002) until senescence (Wingler and Roitsch, 2008). At the earliest stages of plant growth, the perception of sugars influences a number of seedling traits, including primary root growth (Freixes et al., 2002; Yazdanbakhsh et al., 2011) and hypocotyl elongation (Stevenson and Harrington, 2009). As carbohydrate availability fluctuates on a diurnal cycle (Blasing et al., 2005), other metabolic and environmental stimuli are in flux as well (Gibon et al., 2006), and it is therefore necessary for sugar signals to be integrated with other signalling pathways to promote the appropriate developmental responses.

Studies into the effect of sugars on plant growth have revealed a connection between carbohydrates, nutrient status, and primary metabolism (Gibon et al., 2006; Usadel et al., 2008). Transcriptome analysis has uncovered many overlapping and synergistic responses between treatment with sugars and with nutrients, such as nitrate and phosphate, suggesting both similar response pathways as well as interactions between signalling components (Price et al., 2004; Muller et al., 2007). Sugar availability has also been linked to the transcriptional regulation of many components of primary and secondary metabolism, including the oxidative pentose-phosphate pathway (Lejay et al., 2008), the anthocyanin biosynthetic pathway (Solfanelli et al., 2006), and starch metabolism (Sokolov et al., 1998), among others. It is apparent that sugars impact many facets of plant metabolism, and that some of these changes may be regulated at the transcriptional level.

Many plant responses to sugar are mediated by the interaction between metabolic and hormone signalling pathways (Gazzarrini and McCourt, 2001; Rolland et al., 2006; Rook et al., 2006). Forward genetic screens have uncovered sugar-resistant mutants to be novel alleles of known hormone mutants, highlighting the extensive crosstalk between sugar- and hormone-signalling pathways (Zhou et al., 1998; Arenas-Huertero et al., 2000; Laby et al., 2000; Gibson et al., 2001). Crosstalk between hormone pathways is a well-explored phenomenon, and there are many examples of hormone pathways that modify, impinge upon or promote the signalling of other hormones (Jaillais and Chory, 2010; Depuydt and Hardtke, 2011). In instances where
points of crosstalk do exist, interplay between metabolite and hormone signalling may offer a point through which primary metabolism can direct plant growth and development (Falkenberg et al., 2008).

Given the level of redundancy that exists within metabolic and hormone signalling pathways, it is likely that there are pathways that interact with and modify sugar perception that have not been elucidated thus far by classical genetic methods (Smith and Stitt, 2007). As an alternate approach to forward genetic screening, chemical genetics can be used to overcome issues of functional redundancy (McCourt and Desveaux, 2010; Toth and van der Hoorn, 2010). For example, a chemical could bind and inhibit the common active site of a group of redundant proteins (De Rybel et al., 2009), or activate a single member of a family of closely-related receptors (Park et al., 2009). In either scenario, chemical genetics may be used to illuminate biological function that is masked by functional redundancy and likely unattainable through classical forward-genetic approaches.

In addition to overcoming functional redundancy, interconnectivity between biological processes can be inferred by screening for compounds that act synergistically on a phenotypic output (Lehar et al., 2007; Yeh and Kishony, 2007; Owens et al., 2010). Termed “combination chemical genetics”, this approach entails screening chemical libraries in the presence of a compound of interest to uncover secondary chemicals that enhance or negate its effect (Lehar et al., 2008). Hits from these screens can act antagonistically, additively, or synergistically, based on the manner in which their targets interact biologically (Lehar et al., 2007; Lehar et al., 2008). When chemicals act synergistically on a given phenotype, it is believed that the chemicals affect interacting molecular pathways (Yeh et al., 2006; Lehar et al., 2007).

In the present study, a combination chemical genetic screen was used to identify pathways that modify plant responses to sugar. This screen uncovered sulfonamides, known to inhibit tetrahydrofolate (THF) biosynthesis in plants (Prabhu et al., 1998), to act synergistically with sucrose to inhibit etiolated hypocotyl elongation. The pool of folates produced by the THF biosynthetic pathway are required for a variety of metabolic processes and provides the cofactors for the methyl-transfer reactions that constitute one-carbon (C1) metabolism in plants (Hanson and Roje, 2001). Here, the folate inhibitor sulfamethoxazole was used to explore the role of folates in mediating developmental responses to sucrose.
RESULTS

Sulfonamides alter seedling responses to sucrose

Chemicals were screened from the Library of Active Compounds in Arabidopsis (LATCA), and were selected as positive “hits” based on the ability to act synergistically with sucrose to inhibit hypocotyl elongation in dark-grown seedlings (Fig. 1A). Of the 2100 compounds screened, 33 positive hits were reconfirmed in a secondary screen (Supplemental Table S1). Though positive hits were derived from a variety of structural classes and functions, the largest group of successful hits belonged to the sulfonamide family of compounds, which were chosen for further study (Fig. 1B).

Dose-response curves for five sulfonamides showed a saturable, dose-dependent inhibition of hypocotyl elongation that was enhanced by the presence of sucrose (Fig. 1C, Supplemental Fig. S1). The sulfonamides identified in the chemical screen are derivatives of sulfanilamide (Fig. 1B) (Prabhu et al., 1997; Prabhu et al., 1998; Brackett et al., 2004), a compound not found in the LATCA library. These sulfonamides are characterised by the five- or six-membered nitrogen-containing ring attached at the nitrogen of the sulfonamide functional group (Brackett et al., 2004). When tested, sulfanilamide did not exert as strong of an effect compared to other sulfonamides, and required a higher concentration to inhibit hypocotyl elongation (Fig. 1C, Supplemental Fig.S1). This indicates that the substituent group plays a role in determining the efficacy of the sulfonamide. Since sulfamethoxazole (SMX) exerted the strongest effect, it was chosen for use in subsequent experiments. This enabled experiments to be carried out at lower working concentrations of the chemical, which helped reduce any off-target effects of the sulfonamide.

Sulfamethoxazole impinges on sucrose-mediated development through inhibition of Dihydropteroate Synthase (DHPS)

Sulfonamides are a class of compounds known to inhibit the tetrahydrofolate (THF) biosynthetic pathway by competing with para-amino benzoic acid (pABA) for the active site of dihydropteroate synthase (DHPS) (Fig. 2A) (Prabhu et al., 1997; Prabhu et al., 1998; Storozhenko et al., 2007). DHPS is an enzyme that combines dihydropterin and pABA to form dihydropteroate, which is then polyglutamylated and reduced to form THF (Hanson and
Gregory, 2002). THF exists bound to C1 units at various oxidation states, collectively termed folates, and acts as a cofactor in a variety of single-carbon transfer reactions (C1 metabolism) (Hanson and Roje, 2001; Hanson and Gregory, 2002). Though sulfonamides have previously been shown to inhibit DHPS (Prabhu et al., 1997), it is possible that the compounds affected other processes as well, or that the sulfonamides were metabolized to new compounds with alternate modes of action (Zhao et al., 2007). It was therefore necessary to confirm that the phenotypes observed were related to the inhibition of DHPS, rather than secondary effects of the compound. To this end, chemical complementation was performed by supplementing the growth medium with either pABA, or folic acid (FA). The addition of the DHPS substrate (pABA) or product (FA) negated the inhibitory effect of SMX, and rescued hypocotyl elongation (Fig. 2, B and C). This was consistent with the fact that FA supplements the folate pool downstream of DHPS, and therefore compensates for the effect of the compound. In the case of pABA, the effect of SMX was likely reversed by out-competing SMX at the DHPS active site.

To verify that the inhibition of DHPS was modifying sensitivity to sucrose, the effect of SMX was tested on plants with elevated DHPS activity. It was hypothesized that if DHPS is the true target of SMX, then elevating DHPS abundance would confer resistance to the compound. The Arabidopsis genome contains two DHPS isoforms, one mitochondrial (At4g30000) and one cytosolic (At1g69190) (Storozhenko et al., 2007); each was constitutively expressed under the control of the cauliflower mosaic virus (CamV) 35S promoter in planta. Activity of the construct was confirmed by measuring the expression of DHPS in three independently-transformed lines using qPCR (Supplemental Fig. S2A). Although constitutive expression of either isoform attenuated the inhibitory effect of SMX at a lower concentration, higher concentrations of sulfonamide still inhibited hypocotyl elongation at a level similar to wild type (Fig. 3, Supplemental Fig. S2B). The need for higher concentrations of SMX to produce an effect equivalent to wild type in plants over-expressing DHPS was consistent with DHPS being the primary target for the sulfonamide. As a means of explaining the heightened sensitivity to SMX conferred by sucrose, it was hypothesised that sucrose negatively regulates DHPS and that when growing in sucrose the seedlings simply have lower DHPS abundance. To test this, the expression of both isoforms was measured in response to sucrose using qPCR, and it was found that sucrose did not affect transcript abundance of either DHPS isoform (Supplemental Fig. S2C).

As plants that constitutively expressed the wild-type DHPS alleles maintained sensitivity to
higher concentration of SMX, an additional test was undertaken to confirm that the binding of DHPS was necessary to confer sensitivity to sucrose. Plants expressing a microbial sulfonamide-resistant DHPS (sul1 marker) were used to corroborate the mild resistance conferred by over-expressing the wild-type plant DHPS. Consistent with the hypothesis that inhibition of DHPS by the sulfonamide caused hypersensitivity to sucrose, plants harbouring the sul1 marker were highly resistant to SMX (Fig. 3, Supplemental Fig. S2B). These plants exhibited a markedly higher resistance to the sulfonamide, compared to the plant harboring wild-type DHPS alleles. Taken together, the chemical complementation assays and the resistance conferred by enhanced DHPS activity supported the notion that the inhibition of DHPS caused heightened sensitivity to sucrose.

**Changes in transcript abundance of specific auxin signal transduction components occurs in response to the combined action of SMX and sucrose**

The transcriptome-level responses of seedlings to sucrose and SMX were examined by microarray analysis. Concentrations of sucrose and SMX were identified that had no effect on hypocotyl elongation when administered separately (10mM sucrose, 0.2µM SMX), but inhibited elongation when they were present together in the growth media (Fig. 4A). When the relationship between these treatments was tested using a two-way analysis of variance (ANOVA), it was determined that the effect sucrose and SMX exhibited an interaction (P<0.01). These treatments were then used as the basis for a microarray experiment aimed at uncovering changes in the transcriptome that may underpin this interaction.

Complementary statistical analyses were conducted to identify transcripts that may underpin crosstalk between sucrose and SMX. The first approach made use of a two-way ANOVA to identify transcripts that exhibit a sucrose-dependent response to SMX (Interaction). This interaction was significant for nineteen transcripts (Supplemental Table S2), including a number of cell-wall-related enzymes and three members of the Aux/IAA family of transcriptional regulators: IAA3, IAA6, and IAA29. All three Aux/IAAs exhibited decreased transcript abundance in response to co-treatment with sucrose and SMX (Fig. 4B).

A second approach was used to identify transcripts that exhibited synergistic responses to the co-treatment with sucrose and SMX. Linear models were used to identify genes with differential transcript accumulation in which the effect of sucrose and SMX together was at least two-fold.
greater than the additive effect of treatment with sucrose alone and SMX alone (synergy). A total of 117 transcripts met these criteria (Supplemental Table S3). The abundance values for these transcripts were row-normalised and visualised using a heat map, so that trends in transcript accumulation across the four treatments could be observed, irrespective of the absolute abundance (Fig. 4C). Within this group, a fourth Aux/IAA was identified that has previously been shown to regulate auxin responses in the hypocotyl, IAA19/MSG2 (Tatematsu et al., 2004). Similar to the three Aux/IAAs identified by the two-way ANOVA, IAA19 was characterised by decreased transcript abundance in response to the co-treatment of sucrose and SMX (Fig. 4B).

Promoter::GUS fusions were generated to assess the spatial regulation of each of the four Aux/IAAs identified in the microarray analysis. The promoter region of each gene was fused upstream of the uidA sequence, and GUS staining was assessed. IAA6::GUS and IAA19::GUS showed expression patterns that were consistent with the microarray analysis and supported the hypothesis that these Aux/IAAs may regulate responses to SMX and sucrose in the hypocotyl. When grown in the absence of SMX, the IAA6::GUS reporter showed a high level of expression throughout the hypocotyl tissues (Fig. 4D). The presence of SMX restricted expression of the reporter when administered in the presence of sucrose, and the interaction between sucrose and SMX can be seen in the expression of the reporter at the four concentrations used in the microarray analysis (10mM sucrose, 0.2µM SMX). Treating seedlings to both 10mM sucrose and 1µM SMX together completely inhibited expression of the reporter in the hypocotyl, whereas treatment with 1µM SMX in the absence of sucrose still allowed moderate expression. The IAA19::GUS reporter exhibited expression throughout the hypocotyl, similar to the IAA6::GUS line (Fig. 4E). Treatment with SMX did not restrict IAA19::GUS expression independently of the presence of sucrose; however, co-treatment with sucrose resulted in expression of the construct exclusively in the vascular cylinder, and inhibited expression in other tissues of the hypocotyl. Neither the IAA3::GUS nor the IAA29::GUS reporter showed any change in expression within the hypocotyl tissues in response to the treatments (Supplemental Fig. S3). Changes in the expression profiles of IAA3 and IAA29 were observed in other tissues, suggesting that the sucrose-SMX interaction is not limited to the hypocotyl, and may be a more general response throughout the plant.

Interaction between sucrose and SMX is dependent on auxin transport and signal transduction
To test whether auxin transport is necessary to mediate the interaction between sucrose and SMX, plants were grown in the presence of sucrose, SMX and/or the inhibitor of polar auxin transport, naphthylphthalamic acid (NPA) (Morgan, 1964). The presence of 1µM NPA in the growth media attenuated the interaction between sucrose and SMX, and rescued hypocotyl elongation (Fig. 5A). NPA also affected hypocotyl elongation differently depending on whether sucrose was present in the media or not. NPA restricted elongation in the absence of sucrose, yet exerted no effect in its presence, suggesting that despite equal hypocotyl lengths, auxin transport is regulated differentially between these two treatments. These data support the findings of the microarray experiment, and suggest that the interaction between folates and sucrose is dependent on polar auxin transport.

Given that SMX perturbs plant metabolism, it was hypothesised that the compound affects auxin homeostasis, which might explain the changes in Aux/IAA transcript abundance uncovered by the microarray experiment. To test this hypothesis, indole-3-acetic acid (IAA) was extracted from dark-grown seedlings raised in the presence of sucrose, SMX, or a combination of the two treatments, and quantified using liquid chromatography-mass spectrometry (LC-MS). Seedlings were sampled after three or seven days of growth, to assess changes in IAA abundance in response to growth treatments across time. Though IAA levels were lower in samples taken after seven days of growth, there was no change in IAA abundance in response to the growth treatments (Fig. 5B). Auxin was also quantified from seedlings grown in higher concentrations of sucrose and SMX (30mM and 1µM, respectively) to test whether there were concentration-dependent effects not observed at lower concentrations. Similar to the trends observed at lower concentrations, higher levels of sucrose and SMX did not affect IAA abundance (Supplemental Fig. S4). It was concluded that any changes in auxin activity suggested by the transcriptome analysis were occurring independently of auxin levels.

As an alternate hypothesis, the role of auxin signal transduction in mediating crosstalk between sucrose and SMX was explored. This genetic analysis made use of mutants lacking the functional auxin signalling components IAA19 and ARF7. IAA19 has an established role in mediating hypocotyl elongation in response to auxin, and has been shown to interact with the auxin response factor, ARF7 (Tatematsu et al., 2004). A dominant-negative allele of IAA19, msg2, is able to stably block auxin signal transduction, overcoming redundancy in the signalling pathway (Tatematsu et al., 2004). Similarly, the ARF7-dependent signalling pathway can be
shut down by loss-of-function mutations in ARF7. Though many arf mutants do not exhibit mutant phenotype due to functional redundancy, arf7 loss-of-function mutants display reduced auxin sensitivity and tropic responses in the hypocotyl, suggesting that ARF7 may promote specific and partially non-redundant functions in auxin responses (Okushima et al., 2005). Both the dominant-negative msg2 allele and the arf7 loss-of-function mutants were resistant to the inhibition of hypocotyl elongation induced by the action of SMX and sucrose (Fig. 5C). These findings support the hypothesis that the interaction between sucrose and SMX is dependent on auxin signalling, and that phenotypes observed in the hypocotyl are likely related to the activity of the ARF7-dependent auxin response pathway.

Having established that co-treatment with sucrose and SMX perturbed auxin signalling, it was hypothesised that treatment with folic acid would alter seedling responses to auxin. Treatment with exogenous IAA inhibits hypocotyl elongation in dark grown seedlings (Tatematsu et al., 2004). This hypothesis was not supported, in that supplementing the growth media with concentrations of FA up to 50µM did not alter responses to exogenous auxin (Supplemental Fig. S5). Similar findings were observed using lines with enhanced DHPS activity, where heightened DHPS expression had no effect on exogenous auxin (Supplemental Fig. S6), indicating that DHPS itself is not directly involved in auxin signalling. Taken together, it is concluded that though folates may be necessary to maintain normal endogenous auxin signalling, they are not sufficient to alter seedling responses to exogenous auxin.

**Sucrose and SMX promote auxin signalling in vascular tissues of the hypocotyl**

Two auxin-responsive reporter systems were used to assess changes in auxin signalling and accumulation within the hypocotyl: DII-VENUS and DR5. The DII-VENUS system was created by fusing the degron motif of Aux/IAA28 to the VENUS fluorescent protein (Brunoud et al., 2012). Due to the auxin-sensitive degron, the reporter is degraded in the presence of auxin. Consequently, cells with greater auxin concentrations have lower abundance of the reporter (Brunoud et al., 2012). Thus, the DII-VENUS reporter system inversely tracks the distribution of auxin in plant tissues (Brunoud et al., 2012). By contrast, intensity of the reporter in the DR5 system is greater in cells where auxin is being perceived. The DR5 system consists of tandem repeats of the Auxin-responsive element (AuxRE) fused to a minimal promoter (Ulmasov et al., 1997), driving the expression of a reporter gene (Sabatini et al., 1999).
When raised in the absence of sucrose, the DII-VENUS lines showed expression of the reporter in the nuclei of cells along the centre of the hypocotyl, in what appears to be the vasculature. In the absence of sugar, the reporter was observed along the vasculature region irrespective of the presence of SMX. Seedlings grown in the presence of both sucrose and SMX exhibited less expression of the reporter in the vascular region, and at higher concentrations of SMX, expression of the reporter was greatly restricted (Fig. 6A).

Fluorescence intensity was quantified to assess tissue-specific changes in auxin distribution in response to sucrose and SMX. The fluorescence intensity of nuclei from vascular cells relative to those of ground tissue cells was represented as a ratio, where a value of 1 indicated equal distribution across vascular and ground tissues. In the absence of sucrose, the ratio of fluorescence remained close to 1, irrespective of the presence of SMX. This indicated that auxin remained evenly distributed between the tissues of the hypocotyl. SMX caused a decrease in fluorescence in the vasculature, indicated by a reduced ratio of intensity between vasculature and ground tissues. These data support the qualitative assessment that auxin appears to accumulate preferentially in the vascular tissues in response to sucrose and SMX.

Auxin signalling as indicated by the DR5 reporter system was consistent with the trends observed using the DII-VENUS system. Treatment with either SMX or sucrose individually did not induce expression of the DR5::GUS reporter, nor was expression observed in response to the combined treatments of sucrose and SMX at lower concentrations (Fig. 6B). In contrast, expression of the reporter appeared within the vascular region of plants treated with 1µM SMX, but only in the presence of sucrose. Taken together, the reporter systems indicate that the interaction between sucrose and SMX promotes auxin accumulation in the vasculature of the hypocotyl.

Based on these data, a model is proposed in which folate deprivation caused by SMX results in changes to auxin distribution. Treatment with SMX resulted in auxin becoming localised to the vasculature, where IAA19/ARF7-dependent signalling mediates responses to auxin and inhibits hypocotyl elongation (Fig. 6C). The accumulation of auxin in the vasculature is likely the result of transport from other tissues, as evidenced by the attenuation of this affect by treatment with NPA.

DISCUSSION
Combination chemical genetics can be used to overcome genetic redundancy and probe molecular pathways for interaction (Lehar et al., 2007; Lehar et al., 2008). By screening for compounds that act synergistically on a given phenotype, connectivity between targeted pathways can be explored (Lehar et al., 2007; Lehar et al., 2008). As exogenous sucrose exerts a mild restrictive effect on etiolated hypocotyl elongation, it was reasoned that sucrose could be used as the base compound in a combination chemical screen to identify pathways that modify sugar perception. Through this screen, an interaction between folate biosynthesis and sucrose signalling was uncovered. These two pathways appeared to influence auxin signalling through the regulation of a subset of Aux/IAAs. In this sense, the metabolic system has been perturbed by restricting folate biosynthesis, and a small and clearly defined signalling mechanism was changed as a result. Metabolic, hormonal and developmental pathways represent a web of interconnected signalling processes that are integrated to shape growth and development, and it is possible that during normal seedling development folates and sucrose act in concert to shape auxin distribution and sensitivity.

Forward genetic studies have been widely implemented to uncover the genetic basis for plant responses to sugars, and have proven successful in uncovering multiple sugar perception and signalling pathways (Jang et al., 1997; Zhou et al., 1998; Xiao et al., 2000; Rook and Bevan, 2003; Rook et al., 2006). Despite these successes, this approach is often limited by an inability to uncover mutant phenotypes masked by genetic redundancy, where a loss in the activity of one gene through mutation can be compensated by the activity of a related gene (McCourt and Desveaux, 2010; Toth and van der Hoorn, 2010). The Arabidopsis genome contains two isoforms of DHPS (Storozhenko et al., 2007), which may explain why dhps mutants have not been uncovered in screens for sugar-sensitive mutants, as it is possible that either isoform is sufficient to maintain wild-type levels of metabolic flux through the THF biosynthetic pathway. The general inhibition by sulfamethoxazole is believed to restrict the activity of both isoforms equally, and thus promote a phenotypic response while maintaining seedling viability. In this sense, treatment with sulfamethoxazole promoted a phenotype analogous to a double “knockdown” mutation that uncovered a role for DHPS in mediating sucrose responses.

Though this study highlights a novel role for DHPS during seedling development, the effects of the folates in shaping plant growth have been well documented (Storozhenko et al., 2007; Van Wilder et al., 2009; Mehrshahi et al., 2010; Srivastava et al., 2011). A direct connection between
folate and sucrose was observed by Mehrshahi et al. (2010), who uncovered an important role for folylpolyglutamate synthetase (FPGS) across many facets of plant development, including hypocotyl and root elongation (Mehrshahi et al., 2010). Double fpgs2 fpgs3 mutants were seedling lethal, but could be partially rescued by the presence of sucrose (Mehrshahi et al., 2010). To our knowledge, this finding represents the only reported connection directly linking sucrose to the folate pool; however, most studies involving folates were undertaken using light-grown materials, and it is possible that the interaction between folates and photosynthetically-derived sugars underpin some phenotypes observed in these studies.

Treating the aerial tissues of dark-grown seedlings with sucrose can induce profound changes in morphology, such as the initiation of leaf development and root elongation (Roldan et al., 1999). Though the promotion of these traits is generally thought to be underpinned by changes in local auxin distribution (Reinhardt et al., 2003; Grieneisen et al., 2007), no change in total auxin abundance was observed during growth in the presence of sucrose. It is possible that the promotive effect of sucrose on the development of these traits is a result of a redistribution of auxin, rather than de novo biosynthesis. Though auxin has been proposed not to play a dominant role during etiolated hypocotyl elongation (Jensen et al., 1998), our data suggest that the effect of sucrose and SMX on hypocotyl elongation is dependent on polar auxin transport. If sucrose is indeed promoting a change in auxin distribution, this may explain why blocking polar auxin transport attenuated the interaction between sucrose and SMX.

Consistent with the notion that sugars promote auxin activity, sucrose conferred hypersensitivity to exogenous IAA (Supplemental Fig. S5; S6). As sugars can promote the development of traits often associated with auxin, such as leaf development and root elongation (Roldan et al., 1999; Kircher and Schopfer, 2012), this hypersensitivity to auxin may underpin some developmental responses to sucrose. Though the treatments used in this study did not affect total auxin abundance, recent studies highlight the influence of sugars on auxin accumulation during seedling development (Lilley et al., 2012; Sairanen et al., 2012). The hypothesis that sucrose promotes auxin activity in etiolated seedlings is supported by the observation that transcript abundance of many auxin-related signalling components was changed by growth in the presence of sucrose (Supplemental Table S4).

Folate deprivation resulted in changes to auxin distribution, resulting in the inhibition of hypocotyl elongation. Quantifying DII-VENUS expression indicated a preferential accumulation
of auxin in the vascular tissues, relative to ground tissues, in response to SMX. It is possible that folates are especially important in the vasculature, and that phenotypic responses to folate deprivation are a result of tissue-specific signalling. Blocking the effect of SMX with NPA suggests that auxin may be transported from neighbouring tissues to the vasculature in response to the sulfonamide, though the possibility that differential rates of auxin synthesis and metabolism between tissue types is contributing to this distribution cannot be ruled out. Though folates are not sufficient to alter responses to exogenous IAA, they are necessary to maintain normal auxin signalling during etiolated hypocotyl elongation. It may be that auxin inhibits hypocotyl elongation by signalling in the ground tissues, or through signalling pathways not influenced by folates.

Aux/IAAs are core components of auxin signal transduction that negatively regulate auxin signalling through their interaction with Auxin Response Factors (ARFs) (Hagen and Guilfoyle, 2002; Liscum and Reed, 2002; Tiwari et al., 2003). In the presence of auxin, Aux/IAAs are degraded, allowing ARFs to mediate transcription of auxin-responsive genes (Tiwari et al., 2001; Liscum and Reed, 2002). The four Aux/IAAs identified in the microarray analysis have previously been shown to regulate hypocotyl elongation (Kim et al., 1996; Reed et al., 1998; Tatematsu et al., 2004; Koini et al., 2009). IAA3/SHY2 and IAA6/SHY1 were originally identified in genetic screens for suppressors of the long-hypocotyl phenotypes of phyB and hy2, respectively (Kim et al., 1996; Reed et al., 1998). In both cases, dominant alleles were isolated that suppress hypocotyl elongation, and were posited to impinge on light-mediated hypocotyl elongation (Kim et al., 1998; Reed et al., 1998). IAA19/MSG2 was identified in a screen for mutants exhibiting auxin insensitivity in the hypocotyl, and has been shown to mediate tropic responses (Tatematsu et al., 2004; Saito et al., 2007). Though less is known about the specific role of IAA29, a study into plant growth at high temperatures found that IAA29 mediated hypocotyl elongation in response to heat stress (Koini et al., 2009). In a number of different contexts, these Aux/IAAs have been found to regulate hypocotyl development in response to both environmental and endogenous stimuli, suggesting that perhaps they might serve as a point of integration for multiple regulatory inputs.

Transcriptional reporters for each of the four Aux/IAAs identified by the microarray experiment supported the hypothesis that interplay between sucrose and SMX impinged on the expression of these Aux/IAAs. The IAA6::GUS reporter exhibited marked changes in activity throughout the hypocotyl, suggesting this regulator may play a more prominent role in the hypocotyl phenotype.
observed by treatment with SMX and sucrose. Similarly, altered *IAA19::GUS* expression was observed in the ground tissues of the hypocotyl. These changes were similar to those reported by Saito et al. (2007), who observed that during tropic responses, *IAA19* expression was observed in expanding cells of the hypocotyl ground tissue layers, and inhibited in cells that were restricted in elongation (Saito et al., 2007). Perhaps this regulation underpins the short-hypocotyl phenotype, as a restriction of IAA19 expression may result in an inhibition of cell expansion throughout the ground tissues of the hypocotyl. Given that the transcription of Aux/IAAs is activated by auxin (Abel et al., 1995), it may be that the decrease in *IAA6::GUS* and *IAA19::GUS* expression in the ground tissue, and the concomitant increase in *IAA19::GUS* expression in the vascular tissues, indicated a change in auxin distribution (Fig. 6C). In this model, auxin becomes transported from the ground tissue to the vasculature, where IAA19/ARF7-dependent signalling occurs to restrict hypocotyl elongation. The genetic and pharmacogenetic analyses supported this hypothesis, as either the inhibition of polar auxin transport or the inactivation of the IAA19/ARF7 signalling pathway resulted in insensitivity to the sucrose-SMX interaction.

Given the high level of redundancy within the Aux/IAA gene family (Overvoorde et al., 2005), it is possible that all four Aux/IAAs uncovered by the array analysis act in concert to modify plant growth in response to metabolic cues. Changes to Aux/IAA transcript abundance has been observed in response to treatment with hormones (Nakamura et al., 2003), as well as changes to nutrient status (Falkenberg et al., 2008), and may offer a point of crosstalk through which other pathways may modify auxin signalling (Nakamura et al., 2003). By adjusting the abundance of these negative regulators, it may be possible that nutrient status can fine-tune auxin sensitivity. Currently, the mechanism through which SMX promotes changes to Aux/IAA transcript abundance remains unclear, and whether the compound directly regulates Aux/IAA transcript levels, or whether the changes in overall transcript abundance are a result of changed auxin distribution in the seedling, remains to be tested. Future work will explore the mechanisms through which auxin signalling and distribution are regulated in response to nutrient cues during seedling development.

**MATERIALS AND METHODS**

**Plant material and growth conditions**
Wild-type *Arabidopsis thaliana*, ecotype Columbia-0 (Col-0), seeds were obtained from the Nottingham Arabidopsis Stock Centre (NASC); all transgenic lines and mutants are of the Columbia ecotype. Plants harbouring the *DR5::GUS* construct were described previously (Sabatini et al., 1999), as were the dominant-negative *IAA19* allele (msg2) and the *arf7* loss-of-function mutants (Tatematsu et al., 2004; Okushima et al., 2005). All plant materials were grown in Conviron growth cabinets (Conviron) at 21ºC in a 16/8hr photoperiod at 135µmol m⁻² s⁻¹.

**Chemical screen**

Seeds were sown in 96-well microtiter plates containing 200µL 1X Murashige and Skoog (MS) liquid growth media (Murashige and Skoog, 1962), with alternate rows supplemented with 30mM sucrose. Gamborg’s vitamin solution (Sigma-Aldrich) was added (0.1% v/v) after autoclaving the media (Gamborg et al., 1968). Chemicals were drawn from the Library of Active Compounds in Arabidopsis (LATCA), provided by Dr Sean Cutler (UC Riverside), and adjusted to a final concentration of 2.5µM in each well. Approximately twenty seeds were added to each well in the microtiter plate. The plates were wrapped in aluminium foil and cold stratified at 4ºC, after which they were exposed to six hours of light to promote germination, rewrapped in the foil, and then transferred to the growth chamber. After seven days in the growth cabinet, the microtiter plates were unwrapped, the growth media was drawn from the wells, and the seedlings were fixed by adding approximately 250µL Farmer’s Fixative (3:1, ethanol:acetic acid) to each well, ensuring that all seedlings were entirely submersed in the fixative. Upon rehydration using autoclaved dH₂O, seedlings were aligned on agarose plates and photographed using a Canon EOS Rebel XT EF-S 18-55 digital camera. Hypocotyl lengths were measured using ImageJ software (rsbweb.nih.gov/ij/).

**Dose response curves and chemical treatments**

Dose-response curves and chemical complementation analyses made use of a concentrated stock solution of each chemical, dissolved in dimethylsulfoxide (DMSO). Experimental concentrations were adjusted from this stock solution by dilution in sterile liquid growth media. An equivalent volume of DMSO was added to MS medium as a control. Seeds were sterilized as described above, and sown in 24-well microtiter plates in 2mL full strength MS growth media supplemented with the experimental treatments (Murashige and Skoog, 1962). Plates were then wrapped in light-blocking foil, and cold-stratified for three days at 4ºC. After stratification, plates
were unwrapped and exposed to light for six hours to promote germination. Plates were wrapped in foil, and transferred to a conviron growth cabinet (Conviron) at 21°C in a 16/8hr photoperiod at 135µmol m⁻² s⁻¹. After seven days of growth in the cabinet, seedlings were fixed, aligned on agarose plates, and measured as described above.

**Tissue preparation, RNA extraction, and microarray analysis**

Seedlings (Col-0) were grown in 24-well microtiter plates in MS liquid media (Murashige and Skoog, 1962), supplemented with 10mM sucrose, 0.2µM sulfamethoxazole, or both 10mM sucrose and 0.2µM sulfamethoxazole together. Four plates, comprising three experimental treatments and one non-treatment control, were prepared together using sterilized seeds as described above. The four plates were each individually wrapped in light-blocking foil, and cold-stratified at 4°C for three days. The plates were then unwrapped and exposed to light for six hours to promote germination, after which they were wrapped and placed in a conviron growth chamber as described above. Together, these four plates comprise one biological replicate; the experiment was repeated three times to generate three biological replicates for the microarray experiment. After three days of growth, hundreds of seedlings were harvested from each plate under a green safelight, snap frozen in liquid nitrogen, and ground to a fine powder in a mortar and pestle. RNA was extracted using the RNeasy RNA extraction kit (Qiagen), and was precipitated overnight in 3M NaAc at -80°C to achieve concentrations necessary for prehybridization procedures. RNA quality was determined electrophoretically. For each sample, 5µg of total RNA was reverse transcribed (SuperScript II; Invitrogen), labeled and hybridized to the Arabidopsis ATH1 Genome Array according to manufacturer’s protocols (Affymetrix) at the Centre for the Analysis of Genome Evolution & Function at the University of Toronto, Canada. GeneChip data analysis was performed using the Bioconductor suite (Gentleman et al., 2004) in R statistical programming language (R Development Core Team, 2009; [http://www.R-project.org](http://www.R-project.org)) using the affy package (Gautier et al., 2004). All 12 arrays were pre-processed together using GC-robust multi-array analysis (gcrma) (Wu et al., 2004). Expression data were filtered to remove probe sets which reported low transcript abundance and low variance across all arrays (minimum intensity of 100 on at least two arrays, minimum inter-quartile range of 0.5 on the log₂-scale). The pre-processed data were analysed as a 2 X 2 factorial complete randomized ANOVA using the linear model for microarray (limma) package in R (R Development Core Team, 2009). The linear model was parameterized by group means with a manually defined sum-to-zero contrast matrix to test directly for the contrasts of interest: the
main and interaction effects overall, as well as the effect of the co-treatment with sucrose and SMX. A Benjamini-Hochberg false discovery rate of 0.1 was applied to the output of all tests (Benjamini and Hochberg, 1995). Data, description of the experimental design and methods are available for download as GEO accession GSE37484.

**Quantitative RT-PCR**

Total RNA was prepared as described above, except that RNA was treated with DNase to reduce risk of amplification from genomic DNA. A total of 4μg RNA was used for cDNA synthesis from oligo(dT)18 with SuperScript II Reverse Transcriptase (Invitrogen), following the manufacturer’s instructions. Real-time quantitative PCR (qRT-PCR) was performed using the iCycler iQ real-time PCR detection system (Bio-Rad). The relative abundance of cDHPS and mDHPS transcripts were determined by the Pfaffl method (Pfaffl, 2001), using ACTIN2 as a reference gene. A melting curve was performed to assess primer specificity. The primers used were as follows: mDHPS FW5’TGTTGATAATGATACAGTTGC3’, RV5’CTCAAGTAGAGTTCAAGCA3’, cDHPS FW5’GACTATGGGATCAGTGAACC3’, RV5’CCTTTTGTATAATACCAGTCTTT3’, ACT2 FW5’GGCTCCTCTTTACCCAAGGC3’, RV5’CACACCATACCAGAATCCAGC3’.

**Creation of GUS reporter constructs and histochemical analysis**

Four IAA promoter::GUS constructs were created using the Clontech In-Fusion reporter system (Clontech). For each construct, primers were designed to amplify 2kb upstream of the transcriptional start site from genomic Columbia DNA, as follows: IAA3, FW5’CCGGCGCGCCAAGCTCATTTGTTACACGTACGTACG3’, RV5’GATCTACCATGTGCAAGACCTTTAGGAGC3’, IAA6, FW5’CCGGCGCGCCAAGCTTTCTCTCCCTGGAATGCTCT3’, RV5’GATCTACCATGTGCAAGACCTTTAGGAGC3’, IAA19, FW5’CCGGCGCGCCAAGCTATCCTTGAGACTTGTAATATT3’, RV5’GATCTACCATGTGCAAGACCTTTAGGAGC3’, IAA29, FW5’CCGGCGCGCCAAGCTATCCTTGAGACTTGTAATATT3’, RV5’GATCTACCATGTGCAAGACCTTTAGGAGC3’. These PCR products were incorporated into a modified pCambia1390 by homologous recombination, directly upstream of the *uidA* sequence. These plasmids were introduced into E. coli by heat shock for amplification.
of plasmids, which were then extracted using a miniprep kit (Qiagen) and sequenced to ensure accuracy. These plasmids were transformed into *Agrobacterium tumefaciens* strain GV3101 by heat shock method. Arabidopsis ecotype Columbia was then inoculated using the floral dip method (Clough and Bent, 1998). Successful T1 transformants were selected by growth on medium containing 25µg/mL hygromycin.

To facilitate GUS staining, seedlings were immersed in 90% Acetone at -20ºC for 20 minutes, followed by two rinses in 50mM sodium phosphate buffer to remove acetone. GUS expression was assessed by incubating seedlings at 37ºC in GUS buffer containing 50mM sodium phosphate, pH7, 1mg/mL (w/v) 5-bromo-4-chloro-3-indolyl-β-D-glucoronide (X-GLUC; Sigma-Aldrich), 0.5mM K$_3$Fe(CN)$_6$, 0.5mM K$_4$Fe(CN)$_6$, 10mM EDTA. Staining times vary between constructs. *IAA6::GUS* was incubated for 16hrs; *IAA3::GUS, IAA19::GUS, IAA29::GUS* and *DR5::GUS* were incubated for 24hrs. After staining, seedlings were rinsed in autoclaved dH$_2$O, followed by fixation in FAA (50% ethanol, 10% acetic acid, 10% formaldehyde). Tissues were cleared using a chloral hydrate solution (125g chloral hydrate in 50mL 30% glycerol solution) as described previously (Willemsen et al., 1998). Seedlings were observed using an Olympus BX5 microscope (Olympus), and images were captured with a QImaging MicroPublisher 3.3RTV digital camera using QCapture version 2.7 software.

**Confocal microscopy**

The DII-VENUS reporter was visualised using a Leica SP5 confocal microscope (Leica). An argon laser at 514nm was used for excitation while emittance was measured at wavelengths between 518nm and 560nm (Yellow Channel/VENUS) and between 595nm and 690nm (Red Channel/PI). Hypocotyls were counter-stained with propidium iodide by transferring seedlings to the appropriate growth media containing 10µg/mL propidium iodide (PI; Sigma-Aldrich). This was performed under a green safe light, while being careful to disturb the seedlings as little as possible. Images were prepared using Leica Application Suite Advanced Fluorescence - Lite (Leica). Fluorescence was quantified using ImageJ. The intensities of at least ten randomly-selected nuclei were quantified from both the vasculature and the ground tissue. For each nucleus, a neighbouring region was also measured for intensity to subtract background noise. A ratio of intensity of vasculature cells and the ground tissue cells was generated for ten individuals and averaged for each treatment.
Auxin quantification

Indole-3-acetic acid (IAA) was extracted from whole dark-grown seedlings after three or seven days of growth using solid phase extraction as described by Preston et al. (2009) with minor modifications (Preston et al., 2009). Approximately 10-15 mg of frozen tissue was submerged in 1 mL of 80% (v/v) methanol containing 1% (v/v) glacial acetic acid along with 300 pg of d2-IAA (Sigma-Aldrich) as an internal standard. Tissues were disrupted in a TissueLyser II (Qiagen), and stored at 4°C overnight. The samples were centrifuged to remove debris, and the pellet was washed twice. The supernatant was evaporated in a SpeedVac, reconstituted in 1 mL of 1% (v/v) acetic acid, and passed through a pre-equilibrated Oasis HLB column (Waters) according to the manufacturer's instruction. The IAA fraction was washed with 1 mL of water containing 1% (v/v) acetic acid, and eluted with 1 mL methanol containing 1% (v/v) acetic acid. The sample was evaporated in a SpeedVac and reconstituted in 1 mL of water containing 1% (v/v) acetic acid. The resultant was applied to preconditioned Oasis WAX columns (Waters), washed with 1 mL of methanol, and eluted with 1 mL of methanol containing 1% (v/v) acetic acid. The solvent was removed under vacuum and subjected to the liquid chromatograph electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS; Agilent 6410 TripleQuad LC/MS system). An LC (Agilent 1200 series) equipped with a 50 mm x 2.1 mm, 1.8-μm Zorbax SB-Phenyl column (Agilent) was used with a binary solvent system comprising 0.01% (v/v) acetic acid in water (Solvent A) and 0.05% (v/v) acetic acid in acetonitrile (Solvent B). Separations were performed using a gradient of increasing acetonitrile content, and an initial flow rate of 0.2 mL min⁻¹. The gradient was increased linearly from 97% A, 3% B to 50% A, 50% B over 15 min, then acetonitrile content was increased linearly to 98% over 1 min and held for 1 min. This condition was held for an addition 0.5 min with an increased flow rate of 0.3 mL min⁻¹. After 1 min, the initial condition was restored and allowed to equilibrate for 7.5 min for the next analysis. The retention time of IAA and d2-IAA was 11.6 min. MS/MS conditions were as follows: capillary 4.0 kV; source temperature, 100 °C; desolvation temperature, 350 °C; cone gas flow, 0 L/min; desolvation gas flow, 12 L/min; fragmentor, 110; collision energy, 18; polarity, positive; MS/MS transition, 178/132 m/z for d2-IAA and 176/130 m/z for endogenous IAA. A calibration curve was made using MassHunter software.

ACKNOWLEDGMENTS

We are grateful to Joan Ouellette and Thanh Nguyen for excellent technical assistance. We
would also like to thank Hilda Doan and Ray Persaud for assistance with data collection. We are grateful to Dr Yamamoto for sharing previously-published materials. The authors are also very appreciative for the thoughtful and helpful commentary given by the anonymous reviewers.

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FIGURE LEGENDS

Figure 1. Sucrose conferred hypersensitivity to sulfonamides. Chemicals selected as positive hits based on the ability to act synergistically with sucrose to inhibit hypocotyl elongation in dark-grown seedlings (A). Structures of five sulfonamides identified in the chemical screen, plus the core structure sulfanilamide (B). SNA, sulfanilamide; SMZ, sulfamerazine; SMX, sulfamethoxazole; SDZ, sulfadiazine; SMT, sulfameter; SMP, sulfamethoxypyridazine. Dose-response curves of five sulfonamides identified in original chemical screen and the core structure, sulfanilamide, indicate that the effect of sulfonamide on hypocotyl elongation is augmented by the presence of sucrose (C). Data represent the mean ±SE of three biological replicates from a representative experiment; each data point represents at least 36 individuals.

Figure 2. Overview of C1 metabolism in plants. Sulfamethoxazole inhibits dihydropteroate synthase (DHPS), an early step in tetrahydrofolate (THF) biosynthesis (A). Supplementing media with either Folic acid (B) or pABA (C) rescues hypocotyl elongation. Dark-grown seedlings were raised in the presence of 30mM sucrose and SMX, as indicated. Data represent the mean ± SE of three biological replicates from a representative experiment; each data point represents at least 36 individuals. Letters indicate values with statistically significant differences (P<0.05; ANOVA followed by Tukey’s b).

Figure 3. Enhanced DHPS activity attenuated the interaction between sucrose and SMX. Hypocotyls were measured from seven-day-old dark-grown seedlings raised in the presence of sucrose and SMX, as indicated. Plants constitutively expressing either the mitochondrial (35S::mDHPS) or the cytosolic (35S::cDHPS) isoform exhibited mild resistance to the synergistic effect of sucrose and SMX, compared to WT. Plants expressing the bacterial SMX-resistant DHPS (sul1) exhibited greater resistance than plants expressing wild-type plant alleles. Data represent the mean ± SE of three biological replicates from a representative experiment; each data point represents at least 36 individuals. Letters indicate values with statistically significant differences (P<0.05; ANOVA followed by Tukey’s b).

Figure 4. Synergy between sucrose and SMX restricts the expression of a set of Aux/IAAs. Concentrations of sucrose and SMX were determined which when administered independently induced no effect on hypocotyl elongation, yet inhibited it when administered together (10mM sucrose, 0.2µM SMX) (A). Two-way ANOVA indicated an interaction between
the two treatments (P<0.01); these conditions formed the basis of the microarray experiment to
explore transcriptome-level changes that may underpin the interaction. Three Aux/IAAs
identified by ANOVA exhibited sucrose-dependent responses to SMX (IAA3, IAA6, and IAA29).
IAA19 transcript abundance responded synergistically to co-treatment with sucrose and SMX.
Transcript abundance values are displayed in log2 scale (B). Heatmap includes 117 probe sets
that correspond to transcripts identified for which the effect of sucrose and SMX together
(columns 9-12) was at least two-fold greater than the additive effect of treatment with SMX
alone (columns 4-6) and sucrose alone (columns 7-9)(C). Each column corresponds to a
discrete biological sample and all treatments are presented as biological triplicate replicates.
Red indicates higher, and blue indicates lower, levels of transcript abundance. Hierarchical
clustering performed using Pearson correlation coefficients, and data are row-normalized to
identify trends in transcript abundance across treatments irrespective of absolute abundance. In
the absence of SMX, IAA6::GUS and IAA19::GUS expression is observed throughout the
hypocotyl tissues of seven-day-old dark grown seedlings; co-treatment of sucrose and SMX
caused a restriction of reporter expression (D-E). Images were taken along the centre of the
hypocotyl, midway between the shoot apical meristem and the root-hypocotyl junction. Scale
bar, 50µm.

Figure 5. Auxin transport and signalling mediate synergy between sucrose and SMX.
Blocking polar auxin transport with NPA attenuated the synergistic effect of sucrose and SMX
on hypocotyl elongation (A). Data represent the mean ± SE of three biological replicates from a
representative experiment; each data point represents at least 36 individuals (P<0.01; ANOVA
followed by Tukey’s b). Indole-3-acetic acid (IAA) was extracted from three- and seven-day-old
dark-grown seedlings, and quantified by LC/MS (B). No change in auxin abundance was
observed in response to the sucrose or SMX treatments, though seedlings sampled after seven
days of growth exhibited reduced auxin levels, on a per-gram fresh weight (FW) basis. Data
represent the mean ± SD from three independent experiments (P<0.05; ANOVA followed by
Tukey’s b). The IAA19/ARF7 auxin signalling pathway is necessary for the interaction between
sucrose and SMX (C). msg2 is a stable IAA19 allele resistant to degradation in the presence of
auxin (Tatematsu et al., 2004), whereas arf7 is a loss-of-function mutant (Okushima et al.,
2005). In both mutant backgrounds, synergy between sucrose and SMX is attenuated and
hypocotyl elongation is rescued. Data represent the mean ± SE of three biological replicates
from a representative experiment; each data point represents at least 36 individuals. Letters
indicate values with statistically significant differences (P<0.01; ANOVA followed by Tukey’s b).
Figure 6. Co-treatment of sucrose and SMX promotes auxin signalling in the hypocotyl. The DII-VENUS reporter system exhibited high levels of fluorescence in the vascular region of the hypocotyl. Treatment with SMX and sucrose caused a decrease in VENUS fluorescence, yet this effect was not observed when seedlings were treated with SMX or sucrose alone. White numbering indicates the ratio of fluorescence intensity in to vasculature relative to the ground tissues, mean±SD for at least ten individuals. Fluorescence decreased preferentially in the vasculature compared to the ground tissues (A). Scale bar, 100µm. DR5::GUS expression was observed in the hypocotyl vascular region during growth in the presence of both sucrose and SMX, suggesting auxin signalling is enhanced by the combined action of these two treatments (B). Scale bar, 50µm. Images were taken along the centre of the hypocotyl, midway between the shoot apical meristem and the root-hypocotyl junction. A proposed model depicting changes in auxin distribution that may underpin the sucrose-SMX interaction (C). In the absence of SMX, auxin is evenly distributed at low concentrations throughout the hypocotyl. SMX causes auxin accumulation in the vasculature, resulting in hypocotyl inhibition via IAA19/ARF7 signalling.

TABLES

Table I: Number of probe sets with significant main effect or interaction determined by ANOVAa
<table>
<thead>
<tr>
<th>Effect</th>
<th>Number of probe sets</th>
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<tr>
<td>Sulfamethoxazole main effect</td>
<td>12</td>
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<tr>
<td>Sucrose main effect</td>
<td>9292</td>
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<tr>
<td>Sucrose-sulfamethoxazole interaction</td>
<td>19</td>
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\(^a\)Benjamini–Hochberg (BH) adjusted P < 0.1 (Benjamini and Hochberg, 1995).

**SUPPLEMENTAL MATERIAL**

**Supplemental Figure S1. Sucrose confers hypersensitivity to sulfonamides.** Dose-response curves for five sulfonamides identified in the chemical screen, plus the core structure sulfanilamide. Shown here are the effects of the compounds at higher concentrations than
Supplemental Figure S2. Plants with enhanced *DHPS* activity exhibit resistance to the sucrose-SMX interaction. Plants were transformed with constructs expressing either the cytosolic (cDHPS) or mitochondrial (mDHPS) isoform of Dihydropteroate synthase under the control of the 35S cauliflower mosaic virus promoter. Three independently-transformed lines were tested by qPCR to confirm elevated *DHPS* transcript abundance (A). Plant lines expressing either the 35S::cDHPS or 35S::mDHPS construct were resistant to lower concentrations of SMX, yet succumbed to wild-type levels of inhibition at higher concentrations (B). Only the plant line expressing the microbial sulfonamide-resistant allele of DHPS (sul1) was resistant to SMX at all concentrations tested. Note: Only lines 35S::mDHPS-1 and 35S::cDHPS-1 were shown in the main body of the text. The affect of sucrose on DHPS transcript abundance was tested using qPCR; no significant change was observed (ANOVA, p<0.01)(C).

Supplemental Figure S3. Sucrose and SMX do not affect *IAA3::GUS* and *IAA29::GUS* expression in the hypocotyl. Seven-day-old dark-grown seedlings were raised in the presence of sucrose and SMX, as indicated. IAA3::GUS did not exhibit staining in the hypocotyls in during etiolated growth in response to sucrose and SMX (A). IAA29::GUS was expressed faintly throughout the vascular region and was unaffected by treatments. Images were taken along the centre of the hypocotyl, midway between the shoot apical meristem and the root-hypocotyl junction. Scale bar, 50µm.

Supplemental Figure S4. Auxin abundance is unchanged by growth in the presence of sucrose and SMX. Indole-3-acetic acid (IAA) was extracted from seedlings grown in the presence of sucrose and SMX, and quantified by LC/MS. Seedlings were harvested after three or seven days of growth. Though seedlings harvested after seven days of growth tended to contain less auxin on a per-gram fresh weight (FW) basis, the treatments did not induce a significant change in auxin abundance (P<0.05; ANOVA followed by Tukey’s b).

Supplemental Figure S5. Folic acid is not sufficient to alter sensitivity to exogenous auxin. Seedlings were grown in media containing either sucrose, IAA, or a combination of the two supplemented with folic acid (FA) to explore whether treatment with FA alters responses to exogenous auxin. Data represent the mean ± SE of three biological replicates from a representative experiment; each data point represents at least 36 individuals. Letters indicate
values with statistically significant differences (P<0.05; ANOVA followed by Tukey’s b).

**Supplemental Figure S6. Enhanced DHPS activity does not alter the effect of exogenous auxin on hypocotyl elongation.** Transgenic lines with elevated DHPS expression were treated with indole-3-acetic (IAA) acid to explore whether DHPS plays a direct role in response to exogenous IAA. Data represent the mean ± SE of three biological replicates from a representative experiment; each data point represents at least 36 individuals. (P<0.01; ANOVA followed by Tukey’s b).

**Supplemental Table S1.** Complete list of positive hits from initial chemical screen.

**Supplemental Table S2.** Probe sets with significant sucrose-SMX interaction.

**Supplemental Table S3.** Probe sets exhibiting synergy to co-treatment with sucrose and SMX.

**Supplemental Table S4.** Summary of probe sets corresponding to core auxin signalling components with significant sucrose main effect. Included are all ARFs, PINs, Aux/IAAs, and TIR1/ABF receptors that exhibit significantly different transcript abundance as sucrose main effect.
Chemicals added to media containing either 0mM or 30mM sucrose. Seedlings grown in the dark for seven days. Measured hypocotyls to identify chemicals that exhibit sucrose-dependent effects.
Hypocotyl length (cm)

WT  35S::mDHPS  35S::cDHPS  sul1

0μM SMX  0.2μM SMX  1μM SMX  0μM SMX  0.2μM SMX  1μM SMX

0mM Sucrose  10mM Sucrose
No Sucrose-SMX Interaction: Hypocotyl Elongation

Sucrose and SMX Together: Shortened Hypocotyl