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ABSTRACT

Root hairs are protrusions from root epidermal cells with crucial roles in plant soil interactions. Although much is known about patterning, polarity and tip growth of root hairs, contributions of membrane trafficking to hair initiation remain poorly understood. Here, we demonstrate that the trans-Golgi network-localized YPT-INTERACTING PROTEIN 4a and YPT-INTERACTING PROTEIN 4b (YIP4a/b) contribute to activation and plasma membrane accumulation of Rho-of-plant (ROP) small GTPases during hair initiation, identifying YIP4a/b as central trafficking components in ROP-dependent root hair formation.

KEYWORDS: ROP, YIP, Root hair, Secretion, Trans-Golgi network

INTRODUCTION

Root hair formation in plants underlies strict spatial control and in Arabidopsis thaliana (Arabidopsis) root hairs emerge from the basal (root tip-oriented) ends of hair-forming epidermal cells. Members of the Rho-of-plant (ROP) small GTPase family provide early markers for the specific plasma membrane domain marking the incipient site of hair initiation (Molendijk et al., 2001; Jones et al., 2002). The mechanistic framework underlying polar ROP localization has been studied in the context of tip growth (Gu et al., 2003; Hwang et al., 2010; Chang et al., 2013; Huang et al., 2013) and polar positioning of the root hair positioning site (Fischer et al., 2006; Kiefer et al., 2015; Stanislas et al., 2015). Regulation of ROP cycling between a GTP-bound active and a GDP-bound inactive form sequestered in the cytosol represent a key factor.

However, little is known about trafficking to and regulation of ROP accumulation at the root hair initiation site. Here, we report that the trans-Golgi network (TGN)-localized YPT-INTERACTING PROTEIN 4a and YPT-INTERACTING PROTEIN 4b (YIP4a/b) contribute to activation and plasma membrane accumulation of ROPs, identifying YIP4a/b as central trafficking components in ROP-dependent root hair initiation.

RESULTS AND DISCUSSION

We have previously shown that the redundantly acting YIP4a and YIP4b proteins are required for cell elongation and act on secretory trafficking of some proteins and cell wall components via the TGN (Gendre et al., 2011, 2013). Strikingly, our analyses of yip4a yip4b double mutant roots indicated an almost complete absence of root hairs compared with wild type (WT) (Fig. 1A and Fig. S1A,B), with rare or no visible bulges, whereas the single yip4a and yip4b mutants have a similar or slightly higher hair density than wild type, respectively (Fig. S1A,B). Furthermore, expressing YIP4a under its own promoter is sufficient to restore hair formation (Fig. S1A,B). This suggests that both YIP4 proteins are required for hair initiation and act redundantly at an early stage.

In the Arabidopsis root, epidermal cell fate acquisition and subsequently differentiation into hair cells (trichoblast) or non-hair cells (atrichoblast) depends on their position relative to the underlying cortical cells. Mutations that result in a failure to specify trichoblast identity cause the formation of fewer or no hairs and ectopic hair cell specification results in additional hairs. Analyses of the expression pattern of a root hair file-specific marker, the promoter of EXPANSIN7 driving green-fluorescent protein (EXP7::GFP) (Cho and Cosgrove, 2002; Singh et al., 2008) revealed that EXP7::GFP expression starts immediately prior to the formation of the first hair bulges and continues during tip growth, but is absent from non-hair cell files (Cho and Cosgrove, 2002) (Fig. 1B). The pattern of EXP7::GFP expression was not affected by loss of YIP4a and YIP4b function but, unlike in wild type, expression ceased once cells had fully elongated (Fig. S1C). Moreover, the expression of YIP4b driven by the trichoblast-specific COBL9 promoter was observed in trichoblast cell files, as expected (Fig. 1C). These results indicate that it is unlikely that defects in root hair formation in yip4a yip4b are due to a failure of epidermal cell type specification.

Immunostaining employing a YIP4b antibody revealed ubiquitous YIP4b expression in elongating hair and non-hair cells prior to hair formation (Fig. 1C) compared with the absence of signal in the yip4a yip4b double mutant at the same differentiation stage. However, expression of YIP4a or YIP4b from the trichoblast-specific COBL9 promoter in the yip4a yip4b background was sufficient to completely restore root hair development (Fig. 1C and Fig. S1A,B), suggesting that hair cell-specific expression of YIP4 is sufficient for YIP4 function in root hair development.
Following hair cell specification, comes hair initiation marked by bulging at the site of root hair formation, tip-growth and growth cessation (Grierson et al., 2014). As the absence of visible bulges in yip4a yip4b indicated that YIP4s may act at an early stage of root hair development, we investigated the recruitment of ROPs to the basal end of trichoblasts, preceding the formation of the bulge (Molendijk et al., 2001; Jones et al., 2002). We employed an anti-ROP antibody (Kiefer et al., 2015) directed against a conserved epitope in ROP2, ROP4 and ROP6. In cells exiting the meristematic zone, ROPs concentrate into patches at the basal end of the cell before a hair bulge is visible and remain concentrated at the tip of the bulge and in the growing hair (Molendijk et al., 2001; Jones et al., 2002) (Fig. S2A,B). Along the first 900 µm of the root tip, the length chosen to cover hair initiation and was not enhanced by additional downregulation of ROP6 (Molendijk et al., 2001) and DN-ROP2 expression results in fewer and shorter hairs, whereas CA-ROP2 plants produce more and longer hairs than wild type (Jones et al., 2002). To observe the effects of loss of function of ROPs, we examined root hair length and root hair density in rop2 rop4i-8 rop6 single, double and triple mutants (Ren et al., 2016) (Fig. 3A-C). Root hair length, which provides a measure of ROP action on tip growth, was reduced by 20% in rop2 and rop4i-3 (an RNAi line displaying downregulation of ROP4, Fig. S3A) when compared with wild type. Hair length was reduced by about 40% in the rop2 rop4i-8 and by about 70% in the rop2 rop4i-4 rop6 triple line (Fig. 3B). Hair density, scored as the number of hairs per mm root, reflects the action of ROP on hair initiation and was decreased by 22% in rop2 compared with wild type (Fig. 3C) but was not enhanced by additional downregulation of ROP4 or ROP6. However, rop2 rop4i-4 rop6 triple mutants displayed a 40% reduction in hair density compared with wild type. Thus, ROP2, ROP4 and ROP6 act redundantly on root hair length and root hair initiation. Interestingly, the redundancy in ROP signalling in root hair formation differs from that in leaf epidermal patterning, in which ROP2/4 and ROP6 act antagonistically (Fu et al., 2005), but is similar to that observed in petal development, in which they also act redundantly (Ren et al., 2016). ROP4 expression varied between rop4i lines (Fig. S3A), rendering a direct comparison difficult. Nevertheless, rop2 mutation had the strongest individual impact on hair density and length (Fig. 3A-C), while the overall strongest phenotype was observed in the rop2 rop4i-4 rop6 triple mutant, strongly suggesting a role for ROP4 in hair initiation and expansion
The presence of root hairs in rop2 rop4i-4 rop6 suggests that either additional ROPs are involved in root hair development or that residual ROP4 activity, owing to downregulation by RNAi, may account for the presence of the remaining hairs in the triple mutant. Indeed, size and number of the ROP patches were significantly decreased by 18 and 26%, respectively, in rop2 rop4i-4 rop6 compared with wild type (Fig. 3D-F and Fig. S3B) but to a lesser extent than what was observed for yip4a yip4b (24 and 61%, respectively; Fig. 2C,D). These results indicate that the attenuation of ROP at the plasma membrane can significantly contribute to the lack of hairs in yip4a yip4b. Although ROP localization is not completely abolished, the yip4a yip4b mutant is almost hairless. Thus, additional YIP4-dependent factors may be required to proceed to tip growth. Alternatively, the quantity of ROP present in the patch is not sufficient or patches are not maintained long enough to trigger downstream processes in root hair development.

We next analysed the underlying cause for reduced ROP at the plasma membrane in yip4a yip4b. The amount of total ROP protein in wild-type and yip4a yip4b roots was not significantly altered (Fig. S4A,B). Importantly, the plasma membrane levels of EYFP-ROP2 were strongly reduced in yip4a yip4b compared with wild type (Fig. S5A-C), as observed for intrinsic ROP. Thus, reduction of ROP proteins at the plasma membrane in yip4a yip4b is not due to an overall reduction of ROP expression but is likely related to the secretory trafficking function of YIP4s. Whole-cell FRAP analyses bleaching the cell of interest and neighbouring cells expressing EYFP-ROP2 revealed that, after 180 min, fluorescence recovered to 26.8±4.9% in wild type compared with 12.7±1.7% in yip4a yip4b relative to the respective pre-bleach intensities. This substantial decrease in fluorescence recovery further supported the observation that plasma membrane delivery of newly synthesized ROP2 protein requires YIP4a/b function (Fig. 2E and Fig. S5D).

We then investigated whether the decrease of ROP at the plasma membrane could also be due to an attenuation of ROP activation in yip4a yip4b. ROP activity is positively regulated by guanine nucleotide exchange factors (ROP GEFS) that promote the exchange of GDP to GTP. Once active ROPs interact with effectors such as ROP-interactive CRIB-containing proteins (RICs), ROP inactivation relies on GTPase activation protein (ROP GAP) and inactivated ROPs are retrieved from the PM and sequestered in the cytosol by a guanine nucleotide dissociation inhibitor (ROP GDI) (Garcia-Mata et al., 2011). Constitutively activated ROP2 localizes to the PM in Vicia faba guard cells, whereas the dominant-negative variant is mostly cytoplasmic, indicating that the active form is predominantly membrane bound (Jeon et al., 2008). Moreover, reducing ROP activity by mutating FERONIA (FER), a receptor-like kinase interacting with ROP-GEFS, severely affected the number of hairs produced (Duan et al., 2010; Huang et al., 2013). Pull-down experiments in which only activated ROPs are pulled down, employing their effector RIC1 as a bait, revealed 60% less active ROP2 in yip4a yip4b roots compared with the wild type (Fig. 4A; Fig. S6), very similar to the level observed in the spike1 mutant (spk1-4) defective in the ROP-GEF of ROP2 and ROP6 (Fig. 4A) (Ren et al., 2016). However, introducing the ROP-GDI mutant supercentipede1 (scn1-1) or the constitutively active CA-ROP2 form into the yip4a yip4b background did not restore hair growth. Instead, both the multiple bulge phenotype of scn1-1 (Carol et al., 2005), as well as the multiple and ectopic hair phenotype of CA-ROP2, were suppressed in the yip4a yip4b background (Fig. 4B). Thus, genetic approaches that would enhance ROP levels by maintaining ROP at the plasma membrane via blocking its

(Fig. 3A-C). However, neither ROP4 downregulation nor rop6 loss of function individually affected the rop2 mutant root hair phenotype (Fig. 3A-C).
transfer to the cytosol ( scn1-1 ) or by increasing the active pool of ROP ( CA-ROP2 ) did not restore hair initiation. This, combined with a reduced level of active ROP in the yip4a yip4b mutant, strongly suggests that YIP4 function is required for ROP activity and ROP localization to the plasma membrane.

YIP4a and YIP4b localize to the TGN and mediate secretory trafficking from the TGN in Arabidopsis roots. Thus, the hairless phenotype of the yip4a yip4b mutant suggests that YIP4-mediated secretory trafficking plays an important role at an early stage of hair formation. Interestingly, proteomic analysis of a hairless barley mutant revealed that the SECRETION-ASSOCIATED AND RAS-RELATED PROTEIN 1A (SAR1A) GTP-binding protein and a vacuolar ATP synthase subunit B (V-ATPase) are crucial for hair initiation (Janiak et al., 2012). Homologs of these two proteins in Arabidopsis are involved in secretory trafficking and YIP4 colocalizes with V-ATPase at the TGN, supporting a role for TGN-mediated...

Fig. 3. ROP2, ROP4 and ROP6 are required for root hair formation and elongation. (A) Representative images of roots of wild type Col-0 (WT) and the following ROP mutants: rop2, rop4i-3, rop6, rop2 rop4i-8, rop2 rop6, rop4i-5 rop6 and rop2 rop4i-4 rop6. Scale bar: 1 mm. (B) Measure of root hair length (in mm) in all genotypes mentioned above. More than 150 root hairs from at least ten growing roots for each genotype and each replicate (three replicates) were measured (n=450). (C) Hair density measurement (number of hairs per mm) for all genotypes mentioned above. n= 30 roots per genotype (10 for each biological replicate). Data are means±d. analysed using Student’s t-Test (**P<0.01, ***P<0.001). (D-F) Measure of the area (D), length (E) and number of existing patches (F) on a 480 µm region of wild-type and rop2 rop4 rop6 roots, just after the meristem. Data are average±s.d. (n=30 roots for each genotype) analysed using Kolmogorov–Smirnov test (**P<0.01). See Fig. S3B for representative images.

Fig. 4. YIP4a and YIP4b contribute to ROP activation in vitro and to ROP activation of root hair development. (A) Total ROP2 expression in roots of 5-day-old seedlings of wild type, yip4a yip4b and spk1-4 (‘active’) compared with the active population of ROP pulled down by the effector RIC1 (‘total’). Five independent biological experiments were performed and quantifications are given in the graph below (n=5, data are averages±s.d., ***P<0.001; **P<0.01, Student’s t-test, two-tailed). Examples of full blots can be seen in Fig. S6. (B) Root hair phenotype in 5-day-old seedlings of Col-0 (WT), yip4a yip4b, 35S:CA-ROP2 and scn1-1 mutants, and yip4a yip4b CA-ROP2 and yip4a yip4b scn1-1 triple mutants. Scale bar: 500 µm.
secretory trafficking in early hair development. Therefore, a reduced plasma membrane level of ROP or of its activator could be causing the root hair defects observed in yip4a yip4b.

To date, the mechanisms and factors underlying ROP localization at the plasma membrane remain unclear. For example, trafficking of ROP proteins from their site of synthesis on free polysomes in the cytosol to the plasma membrane was originally thought to be a direct process requiring post-translational lipid modification for membrane anchoring. The discovery that post-prenylation modifying enzymes are intrinsic membrane proteins that are restricted to the endoplasmic reticulum (ER) called this view into question (Wright and Philips, 2006; Bracha-Drori et al., 2008). For animals, it has been proposed that Rho-type GTTPases could reach the plasma membrane by two different means: at a slow rate through the vesicle trafficking machinery at the exit of the ER; and at a faster rate by a direct capture at the ER via GDI interaction (Garcia-Mata et al., 2011). In line with this, we detected ROP2/4/6 in SYP61-labelled secretory vesicles both by immunoprecipitation and by label-free quantitative proteomics (Fig. S7A-C). The low abundance of ROP2/4/6 in SYP61-labelled secretory vesicles when compared with YIP4a and YIP4b is consistent with the transient nature of ROP delivery through this compartment and the relatively small amount delivered to the plasma membrane. In contrast, YIP4a and YIP4b are proteins of the SYP61 compartment accumulating there at steady state. Our biochemical data together with the failure of scn1-1 mutations or overexpression of ROP2-CA to increase root hair formation in the yip4a yip4b mutant strongly suggest a role for post-Golgi, TGN-mediated trafficking in ROP localization. Consistent with this hypothesis, interference with TGN function by locking the ARF1 GTTPase either in the GTP- or in the GDP-bound form reduced fluorescence of EYFP-ROP2 at the plasma membrane (Xu and Scheres, 2005). The recent discovery that RAC5/ROP mediates its effector at the TGN before being potentially exocytosed to the plasma membrane (Stephan et al., 2014) further supports the view that ROPs enter post-Golgi traffic, at least from the TGN. Furthermore, EYFP-ROP2 is trapped in brefeldin A (BFA) bodies enriched in TGN vesicles upon BFA treatment, suggesting that ROP trafficking involves the TGN (Xu and Scheres, 2005). However, TGN components that could mediate ROP localization at the plasma membrane have remained enigmatic. The root hair phenotype and the severe attenuation of ROP levels at the plasma membrane in the yip4a yip4b mutant provide evidence that post-Golgi trafficking via YIP4 is crucial for accumulation of activated ROPs in plasma membrane patches (Fig. S8 for schematic model). The functional importance of these findings is corroborated by the root hair density and length defects in mutants with reduced ROP2, ROP4 and ROP6 mutants generated by independent Agrobacterium tumefaciens-mediated transformation using the ROP4-RNAi construct under the native promoter of ROP4 (see Ren et al., 2016) introduced into Col-0, rop2 (SALK_055328C) and rop6 (SALK_091737C), respectively.

The following transgenic fluorescent-protein marker lines were used in the Col-0 background: EXPT::GFP (Cho and Cosgrove, 2002) and ROP2::EYFP-ROP2 (Xu and Scheres, 2005).

**Root hair imaging and measurements**

Five-day-old seedlings grown on half-strength MS agar plates were imaged using Leica MZ9.5 stereo microscope coupled to Leica DC300 camera. For hair density of rop mutants, the number of hairs on the first 4 mm from the tip were manually counted on ten growing roots for each genotype in three independent biological replicates (n=30). For the yip4 mutants and complementation lines, the number of hairs was counted from 5 to 10 mm away from the tip (n=20 seedlings from two biological replicates). Lengths from all hairs present on the first 4 mm from the tip were measured using ImageJ on ten growing roots for each genotype in three independent biological replicates with minimum 150 hairs measured per genotype (n=450). A Student’s t-test was used to check for statistical difference (two-tailed with equal variance).

**Plasmid construction and plant transformation**

1.9 kb of the COBL9 promoter were amplified, as well as the YIP4a and YIP4b ORF using the following primers: COBL9pro-F-BamHI, 5’-atgtagccccacaattagtggcctgatcatgct-3’; COBL9pro-R-AscI, 5’-atgactgcgctgcttctcaagaagaatgaa-3’; YIP4a-ATG-Spel, 5’-atactagttatccgagaaagatt-3’; YIP4a-stop-Nol, 5’-atgccgctgatcatgctgatga-3’; YIP4b-ATG-Spel, 5’-atactagttatccgagaaagatt-3’; and Yip4b-stop-Nol, agctggcgcctgtgctttctccagagaaagttaag-3’.

**Confocal laser-scanning microscopy and immunolabelling**

Fluorescence signals were viewed using a Zeiss LSM 780 confocal laser-scanning system mounted on a Zeiss Axio Observer Z1 inverted microscope, employing a water-corrected C-Apochromat 40× objective, numerical aperture 1.2 (Zeiss). GFP was detected using a 488 nm laser and 493-598 nm emission filter. Arabidopsis root whole-mount immunolabelling employed the protocol previously described (Gendre et al., 2011) with driselase treatment extended to 40 min for enhanced cell wall digestion in elongated root epidermal cells during ROP immunolocalization using rabbit anti-ROP at 1:2500 (Kiefer et al., 2015), rabbit anti-YIP4b at 1:150 (Gendre et al., 2013) and anti-rabbit-DY633 at 1:100 dilution (Agrisera). DY633 was excited using a 633 nm laser and detected using a 638-759 nm emission filter.

**FRAP analysis**

Wild-type and yip4a yip4b plants expressing ROP2::EYFP-ROP2 (Xu and Scheres, 2005) were grown vertically on ½ MS agar plates. Five-day-old seedlings were mounted on a slide and overlaid with a 2 mm layer of MS agar to avoid dehydration. The seedlings were imaged with a Zeiss LSM780 confocal microscope using a water-corrected C-Apochromat 40× objective. For imaging, the microscope was used in Airyscan mode (256 x 256 x 480x), numerical aperture 1.2 (Zeiss). GFP was detected using a 488 nm laser and 493-598 nm emission filter. Arabidopsis root whole-mount immunolabelling employed the protocol previously described (Gendre et al., 2011) with driselase treatment extended to 40 min for enhanced cell wall digestion in elongated root epidermal cells during ROP immunolocalization using rabbit anti-ROP at 1:2500 (Kiefer et al., 2015), rabbit anti-YIP4b at 1:150 (Gendre et al., 2013) and anti-rabbit-DY633 at 1:100 dilution (Agrisera). DY633 was excited using a 633 nm laser and detected using a 638-759 nm emission filter.
membrane intensity was calculated as a percentage of the mean intensity measured per individual cell relative to its pre-bleach intensity. A total of 20 cells from five individual plants (n = 5) per genotype were quantified. The quantified cells had at least two fully bleached cells on either side to rule out recovery from lateral diffusion, etc. in neighbouring cells. Additionally, plasma membrane intensity of two non-bleached adjacent cells per root was determined and used to correct for loss of fluorescence caused by laser excitation during post-bleach image acquisition. Post-bleach intensity values were adjusted to set the intensity immediately after bleaching to zero.

**Pull-down assays**

The ROP pull-down assays were performed as previously described (Ren et al., 2016). For quantification, data were analysed from five biological replicates using ImageJ software with the function of gel intensity analysis. The data are presented as the mean±s.d. (n = 5) of the relative active ROP2 to the total amount of ROP. Statistical analyses were carried out using Student’s t-test.

**Scoring of ROP patches**

After immunolabelling, ROP patches along the first 900 µm region from the quiescent centre (QC), the distance of the first and of the last ROP patch to the QC, as well as the distance of the first root hair from the QC were manually measured with a Zeiss LSM780 using the position setup within the Zen software. The number of ROP patches was manually counted within the 900 µm distance throughout the depth of the entire root tip. As the data were not normally distributed, the significance of differences between distributions was tested using the non-parametric, two-sample Kolmogorov–Smirnov (KS)-test (see above). The quantified values were adjusted to set the intensity immediately after bleaching to zero.

**Image analysis of ROP patches**

ImageJ (image.nih.gov/j) was used to characterize the shape of each patch (area and length) and its associated fluorescence intensity. Maximal projections of confocal sections (z = 35) from two consecutive tiles of 212 µm were each processed equally using a median filter after 8-bit transformation. Particles were isolated using a fluorescence intensity-based threshold and a size exclusion filter to remove all particles that were not considered as a patch but as intracellular punctae or noise. For each patch, surface area and average fluorescence intensity were measured and their maximal length was characterized using the Feret’s diameter. The average and s.d. were calculated based on the pooled samples obtained from three independent replicates with 10 roots each (n = 30). All ROP patches present in the root part analysed were measured and the average of all patches per root represents one sample (n). Significance was tested using the non-parametric, two-sample Kolmogorov–Smirnov (KS)-test (see above).

**Quantification of polar ROP patch position**

Quantification of polar ROP patch position was performed as previously described (Kiefer et al., 2015). In brief, the distance between the basal end of the trichoblast and the basal end of the ROP patch was divided by the total trichoblast length. Measurements were performed on 90 cells (n = 90) pooled and obtained from three experiments, each employing 10 roots from which three cells with clearly distinguishable cell boundaries and ROP patches were measured. Significance of differences between distributions was tested using a non-parametric, two-sample Kolmogorov–Smirnov (KS)-test (P < 0.05).

**Western blot analyses**

Western blots from gels loaded with 40 µg total protein extract per lane quantified by Bradford assay from roots of 7-day-old seedlings were each processed equally using a median filter after 8-bit transformation. Particles were isolated using a fluorescence intensity-based threshold and a size exclusion filter to remove all particles that were not considered as a patch but as intracellular punctae or noise. For each patch, surface area and average fluorescence intensity were measured and their maximal length was characterized using the Feret’s diameter. The average and s.d. were calculated based on the pooled samples obtained from three independent replicates with 10 roots each (n = 30). All ROP patches present in the root part analysed were measured and the average of all patches per root represents one sample (n). Significance was tested using the non-parametric, two-sample Kolmogorov–Smirnov (KS)-test (P < 0.05).

**Quantitative PCR**

Details on DNAse-treated total RNA extraction with an OMEGA Total RNA kit from five-day-old seedlings from three independent experiments, subsequent cDNA synthesis and quantitative PCR performed with a TaKaRa SYBR kit, as well as primer sequence information, can be found in the supplementary Materials and Methods.

**ROP detection in SYP61-positive immuno-purified vesicles**

The immuno-purification procedure for SYP61-positive vesicles, label-free proteomics and LC-MS/MS detection of ROP peptides are described in the supplementary Materials and Methods.

**Accession numbers**

Sequence data used in this research can be found in the TAIR database under the following accession numbers: YIP4a, AT2G18840; YIP4b, AT4G30260; ROP2, AT1G20090; ROP4, AT1G75840; ROP6, AT4G35020.

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**Competing interests**

The authors declare no competing or financial interests.

**Author contributions**


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**Data availability**

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**Supplementary information**

Supplementary information available online at http://dev.biologists.org/lookup/doi/10.1242/dev.168559.supplemental

**References**


