The zebrafish Nodal signal Squint functions as a morphogen

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Secreted morphogens induce distinct cellular responses in a concentration-dependent manner and act directly at a distance[1–7]. The existence of morphogens during mesoderm induction and patterning in vertebrates has been highly controversial, and it remains unknown whether endogenous mesoderm inducers act directly as morphogens[8–10], function locally[9] or act through relay mechanisms[11–12]. Here we test the morphogen properties of Cyclops and Squint—two Nodal-related transforming growth factor-β signals required for mesoderm formation and patterning in zebrafish[13–16]. Whereas different levels of both Squint and Cyclops can induce different downstream genes[14,17–19], we find that only Squint can function directly at a distance. These results indicate that Squint acts as a secreted morphogen that does not require a relay mechanism.

The level of Nodal signalling is a key factor in determining the cell fate induced in responding cells[14,17–21]. We first tested whether Cyclops (Cyc) and Squint (Sqt) can act on responding cells at a distance, and whether the activation of different downstream genes is dependent on concentration and distance. To provide a local source of Sqt or Cyc, *sqt* or *cyc* RNA was injected, together with the lineage tracer fluorescein-dextran, into a single cell of 128–256-cell zebrafish (*Danio rerio*) embryos (Fig. 1a). The injected embryos were fixed 3 h later before the onset of gastrulation (50% epiboly), and analysed by *in situ* RNA hybridization with antisense probes for *no tail/Brachyury* (*ntl*), *goosecoid* (*gsc*), *bhikhari* (*bik*) and *floating head* (*flh*)—known targets of Nodal signalling[13,18,22–23].

We found that *flh*, *ntl* and *bik* were expressed by cells 4–6 (*flh*) and 6–8 (*ntl*, *bik*) cell diameters away from Sqt-producing cells (Fig. 1c, e, m). In contrast, *gsc* was detected only in cells expressing *sqt* and their immediate neighbours (Fig. 1d). This pattern recapitulates the expression of these genes in the zebrafish organizer, where high levels of Nodal signalling are required to activate *gsc*, and lower levels are sufficient to induce expression of *flh*, *ntl* and *bik*[18]. When we reduced the levels of *sqt* tenfold, *ntl* was induced only close to the source and *gsc* was not induced (Fig. 1f, g). Expression of different levels of *cyc* resulted in a similar concentration-dependent induction of *ntl* and *gsc*. High levels induce the expression of both genes, whereas lower levels induce only *ntl* (Fig. 1i, j; and data not shown); unexpectedly, however, we never observed *ntl* or *bik* expression beyond two cell diameters from *cyc*-expressing cells, although the level of *cyc* was high enough to induce *gsc* expression (Fig. 1i, j, l).

Notably, increasing the injected *cyc* RNA to 60 pg did not change the ectopic *ntl* domain, but induced ectopic *gsc* expression in all cells injected with RNA (data not shown). This indicates that more active Cyc protein is made, although western analysis with a haemagglutinin A (HA)-tagged form of Cyc revealed no significant difference in HA–Cyc protein production on injection of 6 pg or 60 pg (data not shown). This result indicates that post-transcriptional mechanisms such as translational efficiency or protein stability might contribute to the range of action of *cyc*.

The expression of *bik* in *cyc*, *sqt* and *cyc;sqt* double mutants is consistent with different ranges for endogenous Cyc and Sqt (Fig. 1o–s). In *cyc* mutants, Sqt is sufficient to activate *bik* 6–8 cells from the margin (Fig. 1q), whereas in *sqt* mutants, Cyc induces *bik* only locally (Fig. 1p). The amino-terminal prodomain of transforming growth factor (TGF)-β signals is thought to interact with extracellular matrix and so may be responsible for the different range of action of Cyc and Sqt[9,24]. We found, however, that the prodomains do not change the range of chimaeric Cyc–Sqt and Sqt–Cyc proteins (Fig. 1h, k), indicating that differences in the

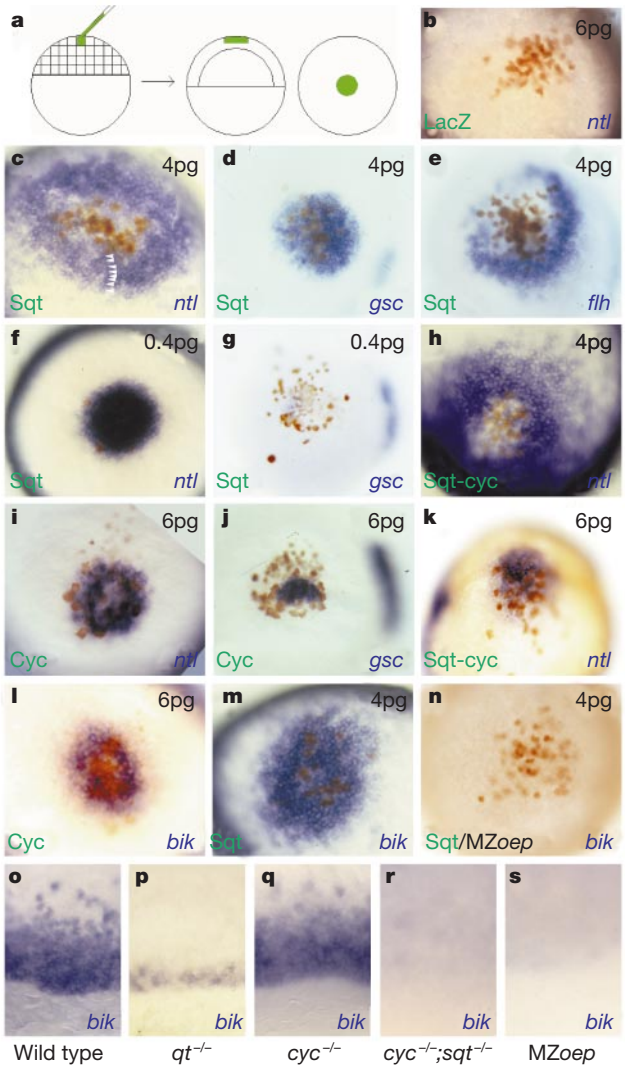

Figure 1 *Squint* has long-range effects, whereas *Cyclops* has short-range effects. **a**, Single-cell injection; RNAs are co-injected with fluorescein-dextran (green) into wild-type embryos at the 128–256-cell stage; embryos are fixed 3 h later at 50% epiboly (left, lateral view; right, animal pole view). *ntl*, *gsc*, *flh* and *bik* expression is examined by whole-mount RNA *in situ* hybridization (blue stain); injected cells are stained brown by an anti-fluorescein antibody. **b**–**n**, Single-cell injections of RNA; all images are animal pole views. **b**, 6 pg of *lacZ* mRNA; no ectopic *ntl* expression is observed ($n = 12/12$). **c**–**e**, 4 pg of *sqt* RNA; *ntl* (**c**, $n = 27/27$) and *flh* (**e**, $n = 18/18$) are expressed in a wider domain than *gsc* (**d**, $n = 34/35$); arrowheads in **c** indicate the domain of ectopic *ntl* expression, about eight cell diameters from *sqt*-expressing cells (about 100 μm). **f**, **g**, 0.4 pg of *sqt* RNA; the ectopic *ntl* domain almost overlaps with the Sqt-producing cells (**f**, $n = 14/15$); no ectopic *gsc* expression is induced (**g**, $n = 13/14$). **h**, 4 pg of *cyc–sqt* RNA ($n = 22/22$); the ectopic *ntl* domain is similar to that induced by *sqt* RNA (**c**). **i**, **j** and **l**, 6 pg of *cyc* RNA; the ectopic *ntl* and *bik* domains almost overlap with the Cyc-producing cells (**i**, $n = 18/20$; **l**, $n = 11/11$) even though the level of Cyc is high enough to induce ectopic *gsc* (**j**, $n = 16/18$). **k**, 6 pg of *sqt–cyc* RNA; the ectopic *ntl* domain is similar to that induced by *cyc* RNA injection ($n = 21/22$). **m**, **n**, 4 pg of *sqt* RNA in wild-type (**m**) and MZ*oep* (**n**) embryos; ectopic *bik* expression is induced up to eight cells away from the Sqt-producing cells in wild type (**m**, $n = 20/20$), but no ectopic *bik* expression is induced in MZ*oep* embryo (**n**, $n = 14/14$). **o**–**s**, *bik* expression at the blastoderm margin at 50% epiboly in different genetic backgrounds, lateral views; *bik* expression is dependent on Nodal signalling around the entire margin.

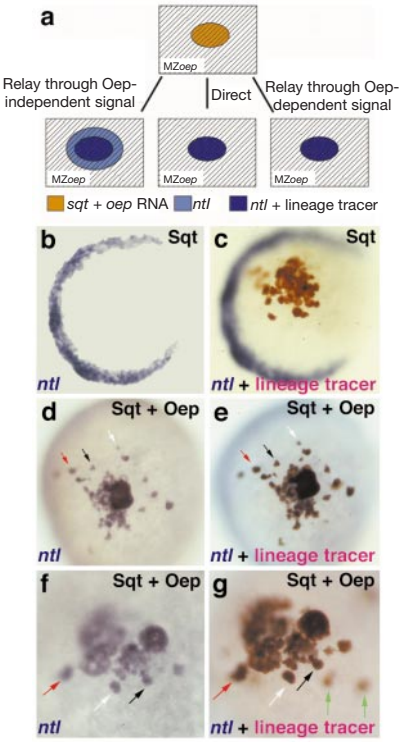

Figure 2 Squint signals to distant cells either directly or through an Oep-dependent relay signal. **a**, Design and predictions for the experiment described in **b**–**g**. **b**–**g**, Single-cell injection into MZ*oep* embryo of 4 pg of *sqt* RNA (**b**, **c**) or 4 pg of *sqt* RNA plus 4 pg of *oep* RNA (**d**–**g**). Note that MZ*oep* mutants express *ntl* at the lateral and ventral margin, but not dorsally (**b**, **c**); **b**, **d**, **f** are *in situ* stainings of *ntl* expression (blue); **c**, **e**, **g** are double stainings of both *ntl* expression (blue) and lineage tracer detected with anti-fluorescein antibody (brown). **b**, **c**, No ectopic *ntl* expression (blue) is detected owing to the inability of MZ*oep* cells to respond to Sqt ($n = 14/14$). **d**–**g**, *ntl* is only expressed by cells injected with *sqt* and *oep* RNA ($n = 19/19$). Arrows of different colours indicate the corresponding cells in **d** and **e**, as well as in **f** and **g**; note that there are two cells marked by the green arrows in **g** that contain the lineage tracer but do not express *ntl*.

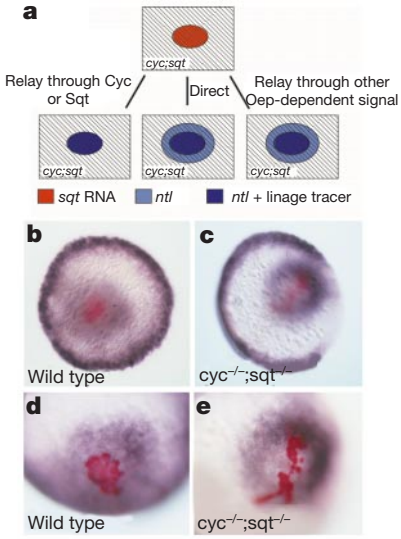

Figure 3 The long-range effect of Sqt does not depend on the induction of the endogenous *cyc* or *sqt* genes. **a**, Design and predictions for the experiments shown in **b**–**e**. **b**–**e**, Single-cell injection of 4 pg of *sqt* RNA plus lineage tracers biotin-dextran and rhodamine-dextran into wild-type (**b**, **d**, $n = 18/18$) and *cyc;sqt* double mutant embryos (**c**, **e**, $n = 12/12$) at the 128–256-cell stage. **b**, **c**, Focus is on the expression domain of *ntl* in marginal cells; *cyc;sqt* double mutants were distinguished from sibling embryos (**b**) by the lack of *ntl* expression on the dorsal side (**c**). **d**, **e**, High-magnification views of the embryos in **b** and **c**; the lineage tracer biotin-dextran was detected with the ABC kit from Vectastain (red).

mature region contribute to the different ranges of action. Together, these results indicate different effective ranges for Sqt and Cyc.

Given the dynamic cell movements during early embryogenesis, the apparent long-range effect of Sqt might be a result of the displacement of cells that initiated expression of *ntl* because early on they were adjacent to *sqt*-expressing cells[25]. Although the short-range effect of Cyc argues against this possibility, we tested it by transplanting labelled wild-type cells at a distance from the *sqt*-expressing cells. To make sure that these two cell populations never got within close proximity of each other, we monitored their relative positions after transplantation. We found that transplanted cells that maintained their distance from the Sqt source expressed *ntl*, as did their neighbouring host cells (Supplementary Information Fig. 1). Thus, cell movement does not cause the long-range effect of Sqt.

Two models can explain the long-range effect of Sqt: Sqt might act as a morphogen and directly activate target gene expression at a distance; or Sqt might activate a relay signal, which then acts on distant cells. Both mechanisms have been invoked previously to account for the long-range effects of non-endogenous TGF-β signals[8–12]. To assay the range of Sqt's direct action, we made use of the observation that cells that lack the activity of the EGF-CFC protein One-eyed pinhead (Oep) cannot perceive Nodal signals[22]. Oep is an extracellular cofactor required cell-autonomously for the reception of Nodal signals[22,26–28].

Maternal-zygotic mutants for *oep* (MZ*oep* embryos) have an identical phenotype to *cyc;sqt* double mutants and are unresponsive to Nodal signals[22]. These mutants are not impaired in signalling for fibroblast growth factor (FGF), bone morphogenetic protein (BMP) or Activin[22], but none of the known Nodal-responsive genes (*bik*, *ntl*, *gsc*, *flh*, *antivin*, *pitx2*, *cyc*) can be activated by overexpression of Sqt in MZ*oep* mutants, and ubiquitous or local expression of Sqt has no phenotypic effects in MZ*oep* mutants[22] (Figs 1n, 2c; and data not shown). Using these MZ*oep* mutants, we tested whether Sqt acts directly at a distance or indirectly through a relay signal.

We first juxtaposed Sqt-responsive cells with Sqt non-responsive cells (*oep* mutant) to test whether an Oep-independent relay signal such as FGF[12] is involved in the long-range effect of Sqt (Fig. 2). We injected both *sqt* and *oep* RNAs into a single cell of 128–256-cell MZ*oep* embryos. *oep* RNA injection allows the injected cells to transduce the Sqt signal. If Sqt can induce a relay signal whose transduction is Oep independent and activates target genes such as *ntl*, then *ntl* would be expressed not only in the injected cells but also in the surrounding *oep* mutant cells (Fig. 2a). But if Sqt acts as a morphogen or relays through signals whose transduction is dependent on Oep, then *ntl* expression would be observed only in cells expressing Oep. Consistent with the second scheme, we found that only cells containing both Oep and Sqt expressed *ntl* (Fig. 2d–g). No non-autonomous induction of *ntl* was observed.

To test whether the long-range effect of Sqt is a result of propagated induction of *cyc* or *sqt*, we analysed *ntl* expression induced by Sqt in *cyc;sqt* double-mutant embryos (Fig. 3a). We found that the domain of *ntl* expression induced in these embryos (Fig. 3c, e) is very similar to that in wild-type embryos (Fig. 3b, d), suggesting that the long-range effect of Sqt does not depend on the induction of the endogenous *cyc* or *sqt* genes.

These results are consistent with the idea that Sqt acts as a morphogen, but it is also possible that Sqt may relay through an unknown signal whose transmission is Oep dependent (Fig. 4a). To address this possibility, we injected *sqt* RNA into a single cell of

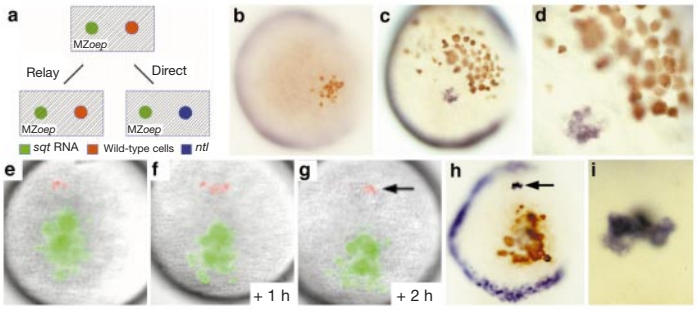

Figure 4 Squint signals directly to distant cells. **a**, Experimental design and predictions. **b**, Control; transplantation of wild-type cells (brown) into MZ*oep* embryo, no ectopic *ntl* expression is observed ($n = 14/14$). Note that MZ*oep* mutants express *ntl* at the lateral and ventral margin, but not dorsally. **c**–**i**, Single-cell injection of 4 pg of *sqt* RNA and fluorescein-dextran (green) into MZ*oep* embryo, followed by transplanting wild-type donor cells labelled with biotin-dextran and rhodamine-dextran (red) at a distance to the *sqt*-injected cells (green) at the 1,000–2,000-cell stage; two examples (**c**–**d** and **e**–**i**) are shown. **c**, **d**, Note ectopic *ntl* staining (blue) at a distance from the *sqt*-expressing cells (brown) ($n = 7/18$). **e**–**g**, Images of the embryo in **h** and **i** after transplantation; **e** was taken shortly after transplantation; **f** and **g** were taken 1 h (**f**) and 2 h (**g**) after **e**. **h**, **i**, Transplanted cells (arrow in **g**) stained with *ntl* probe (arrow); **i**, High-magnification view of the *ntl*-expressing cells in **h** ($n = 6/12$).

MZ*oep* mutants and then transplanted several wild-type cells at a distance to the *sqt*-expressing cells. We also followed the movement of both *sqt*-expressing mutant cells and transplanted wild-type cells to ensure that they were not in close proximity between the time of transplantation and fixation (Fig. 4e–g).

Owing to the lack of Oep function in *sqt*-expressing and neighbouring cells, Sqt cannot activate the Nodal signalling pathway in these cells and thus no relay signal can be produced. If Sqt acts by a relay mechanism, the transplanted wild-type cells would not be able to express *ntl*. But if Sqt activates gene expression directly at a distance, wild-type cells should be able to receive the Sqt signal and express *ntl*. Consistent with the latter possibility, we found that wild-type cells in MZ*oep* mutant embryos can express *ntl* in response to a distant source of Sqt. This result suggests that Sqt activity produced in unresponsive (*oep* mutant) cells can traverse a field of unresponsive (*oep* mutant) cells to elicit an effect in distant responsive (wild-type) cells. These results show that the long-range effect of Sqt is direct, requiring no relay signal (Fig. 4c–i).

The idea of locally secreted signals that act as morphogens and directly pattern a tissue by eliciting distant responses has been an attractive mechanism to explain many developmental events[1–10]. The potential of vertebrate mesoderm inducers to act as morphogens has been debated for a long time. Seemingly contradictory analyses of non-endogenous TGF-β signals have supported conflicting models. Elegant studies in *Xenopus* explants have revealed the potential of Activin to act as a long-range morphogen[8–10]. In contrast, *in vivo* studies in zebrafish have suggested that Activin acts only locally and induces FGF as a relay signal[12]. Similarly, Xnr2 and TGF-β1 have been suggested to act only at short range[9,11]. Our studies using the *in vivo* mesoderm inducers Cyc and Sqt now indicate that both short- and long-range mechanisms can operate during mesoderm induction and patterning. Notably, our results indicate that Sqt may act as a secreted morphogen: first, different levels of Sqt induce different downstream responses in a concentration- and distance-dependent way[14,17–19] (Fig. 1); second, Sqt can act directly at a distance (Figs 2–4). These results indicate that Squint acts as a secreted morphogen that does not require a relay mechanism. □

Methods

Single-cell injection

The lineage tracer fluorescein-dextran was co-injected with the RNA to mark the injected cell and its progeny. We synthesized *sqt*, *cyc* and *oep* sense, capped mRNAs as described[18]. RNA *in situ* hybridization and anti-fluorescein antibody staining were performed as described[22].

Genotyping

After photography, embryos were washed twice in benzyl benzoate : benzyl alcohol (2:1), two or three times in 100% methanol and once in 100% ethanol. We extracted genomic DNA using the DNeasy Tissue Kit (Qiagen). After extraction, genomic DNA was precipitated and resuspended in 50 μl H_2O. Five microlitres of the genomic DNA was used as a template in each polymerase chain reaction (PCR). Primers and conditions for genotyping sqt^{cz35} and cyc^{m294} have been described[13].

Chimaera construction

Cyc–Sqt and Sqt–Cyc fusion proteins were constructed using PCR amplification. The junction sequence of Cyc–Sqt is RRGRRNHRT, in which the underlined sequence is the C terminus of the Cyc prodomain and the rest is the N terminus of the Sqt mature peptide. The junction sequence for Sqt–Cyc is RRHRRGPPVRR, in which the underlined sequence is the N terminus of the Cyc mature peptide and the rest is the C terminus of the Sqt prodomain.

Transplantation and triple labelling

Donor embryos were injected with biotin-dextran and rhodamine-dextran at the 1–4-cell stage. We carried out transplantation as described[22]. After *in situ* hybridization and detection of fluorescein-dextran, the biotin-dextran labelled donor cells were detected with the alkaline phosphatase substrate kit 1 (Vector Laboratories, Inc.).

Imaging

Fluorescent and Nomarski images were taken after mounting the embryo in 2.5% methyl cellulose. Images were acquired as described[18].

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1. Turing, A. M. The chemical basis of morphogenesis. *Phil. Trans. R. Soc. Lond. B.* **237,** 37–72 (1952).
2. Wolpert, L. Positional information and the spatial pattern of cellular differentiation. *J. Theor. Biol.* **25,** 1–47 (1969).
3. Nellen, D., Burke, R., Struhl, G. & Basler, K. Direct and long-range action of a DPP morphogen gradient. *Cell* **85,** 357–368 (1996).
4. Lecuit, T. *et al.* Two distinct mechanisms for long-range patterning by Decapentaplegic in the *Drosophila* wing. *Nature* **381,** 387–393 (1996).
5. Zecca, M., Basler, K. & Struhl, G. Direct and long-range action of a wingless morphogen gradient. *Cell* **87,** 833–844 (1996).
6. Neumann, C. J. & Cohen, S. M. Long-range action of Wingless organizes the dorsal–ventral axis of the *Drosophila* wing. *Development* **124,** 871–880 (1997).
7. Neumann, C. & Cohen, S. Morphogens and pattern formation. *BioEssays* **19,** 721–729 (1997).
8. Gurdon, J. B., Harger, P., Mitchell A. & Lemaire, P. Activin signalling and response to a morphogen gradient. *Nature* **371,** 487–492 (1994).
9. Jones, C. M., Armes, N. & Smith, J. C. Signalling by TGF-β family members: short-range effects of Xnr-2 and BMP-4 contrast with the long-range effects of activin. *Curr. Biol.* **6,** 1468–1475 (1996).
10. McDowell, N., Zorn, A. M., Crease, D. J. & Gurdon, J. B. Activin has direct long-range signalling activity and can form a concentration gradient by diffusion. *Curr. Biol.* **7,** 671–681 (1997).
11. Reilly, K. M. & Melton, D. A. Short-range signaling by candidate morphogens of the TGF beta family and evidence for a relay mechanism of induction. *Cell* **86,** 743–754 (1996).
12. Rodaway, A. *et al.* Induction of the mesendoderm in the zebrafish germ ring by yolk cell-derived TGF-β family signals and discrimination of mesoderm and endoderm by FGF. *Development* **126,** 3067–3078 (1999).
13. Feldman, B. *et al.* Zebrafish organizer development and germ-layer formation require nodal-related signals. *Nature* **395,** 181–185 (1998).
14. Sampath, K. *et al.* Induction of the zebrafish ventral brain and floorplate requires cyclops/nodal signalling. *Nature* **395,** 185–189 (1998).

15. Rebagliati, M. R., Toyama, R., Haffter, P. & Dawid, I. B. cyclops encodes a nodal-related factor involved in midline signaling. *Proc. Natl Acad. Sci. USA* **95,** 9932–7 (1998).
16. Schier, A. F. & Shen, M. M. Nodal signalling in vertebrate development. *Nature* **403,** 385–389 (2000).
17. Erter, C. E., Solnica-Krezel, L. & Wright, C. V. Zebrafish nodal-related 2 encodes an early mesendodermal inducer signaling from the extraembryonic yolk syncytial layer. *Dev. Biol.* **204,** 361–372 (1998).
18. Gritsman, K., Talbot, W. S. & Schier, A. F. Nodal signaling patterns the organizer. *Development* **127,** 921–932 (2000).
19. Thisse, B., Wright, C. V. & Thisse, C. Activin- and Nodal-related factors control antero-posterior patterning of the zebrafish embryo. *Nature* **403,** 425–428 (2000).
20. Jones, C. M., Kuehn, M. R., Hogan, B. L., Smith, J. C. & Wright, C. V. Nodal-related signals induce axial mesoderm and dorsalize mesoderm during gastrulation. *Development* **121,** 3651–3662 (1995).
21. Agius, E., Oelgeschlager, M., Wessely, O., Kemp, C. & De Robertis, E. M. Endodermal Nodal-related signals and mesoderm induction in *Xenopus*. *Development* **127,** 1173–1183 (2000).
22. Gritsman, K. *et al.* The EGF-CFC protein one-eyed pinhead is essential for nodal signaling. *Cell* **97,** 121–132 (1999).
23. Vogel, A. M. & Gerster, T. Promoter activity of the zebrafish bhikhari retroelement requires an intact activin signaling pathway. *Mech. Dev.* **85,** 133–146 (1999).
24. Ruppert, R., Hoffmann, E. & Sebald, W. Human bone morphogenetic protein 2 contains a heparin-binding site which modifies its biological activity. *Eur. J. Biochem.* **237,** 295–302 (1996).
25. Pfeiffer, S., Alexandre, C., Calleja, M. & Vincent, J. P. The progeny of wingless-expressing cells deliver the signal at a distance in *Drosophila* embryos. *Curr. Biol.* **10,** 321–324 (2000).
26. Schier, A. F., Neuhauss, S. C., Helde, K, A., Talbot, W. S. & Driever, W. The one-eyed pinhead gene functions in mesoderm and endoderm formation in zebrafish and interacts with no tail. *Development* **124,** 327–342 (1997).
27. Zhang, J., Talbot, W. S. & Schier, A. F. Positional cloning identifies zebrafish one-eyed pinhead as a permissive EGF-related ligand required during gastrulation. *Cell* **92,** 241–251 (1998).
28. Strähle, U. *et al.* one-eyed pinhead is required for development of the ventral midline of the zebrafish (*Danio rerio*) neural tube. *Genes Funct.* **1,** 131–148 (1997).

Supplementary information is available on *Nature*'s World-Wide Web site (http://www.nature.com) or as paper copy from the London editorial office of *Nature*.

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MOR1 is essential for organizing cortical microtubules in plants

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Microtubules orchestrate cell division and morphogenesis, but how they disassemble and reappear at different subcellular locations is unknown. Microtubule organizing centres are thought to have an important role, but in higher plants microtubules assemble in ordered configurations even though microtubule organizing centres are inconspicuous or absent. Plant cells generate highly organized microtubule arrays that coordinate mitosis, cytokinesis and expansion. Inhibiting microtubule assembly prevents chromosome separation[1], blocks cell division[2] and impairs growth polarity[3]. Microtubules are essential for the formation of cell walls, through an array of plasma-membrane-associated cortical microtubules whose control mechanisms are unknown. Using a genetic strategy to identify microtubule organizing factors in *Arabidopsis thaliana*, we isolated temperature-sensitive mutant alleles of the *MICROTUBULE ORGANIZATION 1* (*MOR1*) gene. Here we show that MOR1 is the plant version of an ancient family of microtubule-associated proteins[4]. Point mutations that substitute single amino-acid residues in an amino-terminal HEAT repeat impart reversible temperature-dependent cortical microtubule disruption, showing that MOR1 is essential for cortical microtubule organization.

In most plant cells that display diffuse rather than tip growth, microtubules localize to the cortical cytoplasm perpendicular to the major axis of expansion. Microtubules and cellulose microfibrils often have similar orientation patterns in elongating cells[5] and it is generally accepted, but not proven, that cortical microtubules control the alignment of cellulose microfibrils[6]. Identifying the factors that organize microtubule arrays at the periphery of plant cells is a necessary step towards understanding the mechanisms that underlie wall deposition and, hence, plant morphogenesis.

To identify factors regulating cortical microtubule organization in plant cells, we used immunofluorescence microscopy to screen chemically mutagenized seedlings of *A. thaliana* for aberrant microtubule patterns. One mutant locus, *mor1*, causes temperature-sensitive cortical microtubule shortening and disorganization (Fig. 1) and consequent morphological defects. We used ecotype-specific markers to identify *MOR1* as a gene of around 14 kilobases (kb) that encodes a protein with a predicted relative molecular mass of 217,000 (M_r 217K) (Fig. 2a) that has significant deduced amino-acid sequence similarity to human TOGp[7], *Xenopus* MAP215

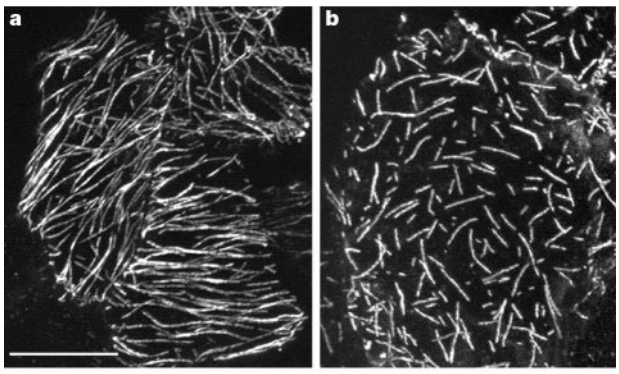

Figure 1 Mutations in the *MOR1* gene cause temperature-dependent microtubule disruption. We used anti-tubulin immunofluorescence to label cortical microtubules in epidermal cells of the first true leaf of 21-day-old seedlings after incubating seedlings at 29 °C for 2 h before fixation. **a**, Wild type. **b**, *mor1-1* homozygote. Scale bar, 25 μm.

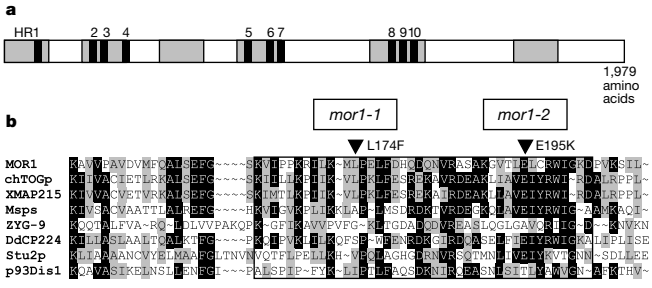

Figure 2 MOR1 protein structure. **a**, Schematic representation with shaded boxes indicating conserved domains between MOR1, TOGp, XMAP215, MSPS and DdCP224. The shorter ZYG-9 and yeast proteins share the first five and first four regions of homology, respectively. Black stripes show putative HEAT repeats (HR) in relation to these conserved domains. **b**, Deduced amino-acid sequence comparison of MOR1's HEAT repeat-1 with equivalent repeats in homologues. Black-shaded residues indicate identity, grey-shaded residues indicate similarity (40% threshold), outlined box indicates HEAT repeat. Mutations altering MOR1 amino-acid residues are indicated by arrowheads.

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