tocrystalline anisotropy is expected between [001] and [100] directions for CoFe₂O₄, the experimentally measured magnetic anisotropy should arise primarily from the magnetoelastic coupling. The first source of stress is the mismatches between the CoFe₂O₄ and BaTiO₃ lattices at the grain boundary (Δε₉₂₆₁). High-resolution TEM images (fig. S1) show that this mismatch is accommodated by the formation of interface dislocations. The second source of stress is the lattice distortion in the CoFe₂O₄ as a consequence of the cubic-tetragonal structural distortion in the BaTiO₃ matrix below the ferroelectric Curie temperature (ε₀₀₁). This contribution decreases the compression along the axis of the CoFe₂O₄ nanopillar. This compressive strain in the CoFe₂O₄ lattice can be related to the magnetic anisotropy through its magnetostrictive effect. The stress in the CoFe₂O₄ is given by σ₀₀₁ = Y₀₀₁ε₀₀₁, in which Y is Young’s modulus (~170 GPa (16)) and ε₀₀₁ is the strain along the [001] direction. The magnetoelastic energy associated with it is \( E_{\text{magnetoelastic}} \equiv \sigma \cdot \varepsilon = \frac{1}{2} \cdot \frac{Y}{\rho} \varepsilon^2 \), where \( \varepsilon \) is the magnetostrictive coefficient of CoFe₂O₄ [taken to be \( \varepsilon \equiv \frac{1}{2} \cdot 5 \times 10^{-6} \) (16)], leading to a magnetoelastic anisotropy energy of \( 5.95 \times 10^6 \) erg/cm³. The anisotropy field is given by \( H_{\text{stress}} \equiv 2\varepsilon / M_0 = 34 \) kOe, which is comparable to our experimentally observed value of \( \approx 35 \) kOe.

Results of temperature-dependent magnetization measurements (Fig. 3D, red curve) show that the change in the magnetic order parameters in the self-assembled nanostructure is manifest as a distinct drop in the magnetization of \( \approx 16 \) emu/cm³ (~5% of magnetization at a 100-Oe external field) around the ferroelectric Curie temperature (\( T_C \approx 390 \) K). At temperatures higher than \( T_C \), the tetragonal distortion in the BaTiO₃ lattice decreases this compression in the CoFe₂O₄. Because CoFe₂O₄ has a negative magnetostriction, it results in a reduction of the moment, as observed in our experiments. The change of magnetization near \( T_C \) can be estimated as \( \Delta M / M \approx Q^2 \varepsilon (d \varepsilon / dM) / M \) from a minimization of free energy (Eq. 4). However, quantitative estimation is difficult due to a lack of information about temperature dependence of \( \varepsilon_0 (M) \). For comparison, the temperature dependence of magnetization at 100 Oe (black curve in Fig. 3D) for a CoFe₂O₄-BaTiO₃ multilayer sample with a layer thickness of \( \approx 30 \) nm shows negligible change around the ferroelectric Curie temperature. This can be understood as a consequence of the in-plane piezo-deformation in the multilayer structure being clamped by the substrate, thus precluding any deformation in the magnetic layer. This also proves that the coupling is dominated by elastic interactions in two-phase nanostructures.

In summary, an epitaxial CoFe₂O₄-BaTiO₃ ferroelectromagnetic nanocomposite was made by a simple self-assembly technique. This system shows a strong coupling of the order parameters through the heteroepitaxy of the two lattices. This approach is general—we have been able to create similar structures of other spinel-perovskite systems such as cobalt ferrite/bismuth ferrite and cobalt ferrite/lead titanate—and as such should impact a broad range of materials research.

References and Notes

Crosstalk Between the EGFR and LIN-12/Notch Pathways in C. elegans Vulval Development

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The Caenorhabditis elegans vulva is an important paradigm for cell-cell interactions in animal development. The fates of six vulval precursor cells are patterned through the action of the epidermal growth factor receptor–mitogen-activated protein kinase (EGFR-MAPK) inductive signaling pathway, which specifies the 1° fate, and the LIN-12/Notch lateral signaling pathway, which specifies the 2° fate. Here, we provide evidence that the inductive signal is spatially graded and initially activates the EGFR-MAPK pathway in the prospective 2° cells. Subsequently, this effect is counteracted by the expression of multiple new negative regulators of the EGFR-MAPK pathway, under direct transcriptional control of the LIN-12-mediated lateral signaling.

The six vulval precursor cells (VPCs) are consecutively numbered P3.p to P8.p (Fig. 1A). Without activation of either the inductive signal causes the 1° fate, whereas a high level of either the inductive or lateral signaling pathways, P3.p, P4.p, and P8.p adopt the 3° fate, believed to be as a result of inhibitory influences from the hypodermal syncytium. Vulval development [reviewed in (1)] is initiated when LIN-3, an EGF-like signal produced by the gonad, activates the EGR homolog LET-23 in the central VPC, P6.p. Activated LET-23, by means of a canonical Ras-MAPK cascade, causes P6.p to adopt the 1° fate and transcribe genes encoding the lateral signal (2). The lateral signal activates the receptor LIN-12/Notch in the two neighboring VPCs, P5.p and P7.p, causing them to adopt the 2° fate. Without activation of either the inductive or lateral signaling pathways, P3.p, P4.p, and P8.p adopt the 3° fate, believed to be as a result of inhibitory influences from the hypodermal syncytium.

Genetic and cell-ablation experiments have led to different models of inductive signaling (1). One model proposes that the inductive signal forms a morphogen gradient from the anchor cell, such that a high level of inductive signal causes the 1° fate, whereas a lower level helps specify the 2° fate (3). An alternative model proposes that VPC patterning is achieved by sequential induction, such that the inductive signal activates LET-23 only in P6.p, leading to a lateral signal that then induces P5.p and P7.p to adopt the 2° fate and transcribe genes encoding the lateral signal (4). The lateral signal activates the receptor LIN-12/Notch in the two neighboring VPCs, P5.p and P7.p, causing them to adopt the 2° fate. Without activation of either the inductive or lateral signaling pathways, P3.p, P4.p, and P8.p adopt the 3° fate, believed to be as a result of inhibitory influences from the hypodermal syncytium.

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Fig. 1. Signaling events involved in VPC specification and evidence that the inductive signal is graded. (A) Net effect of inductive and lateral signaling during VPC specification. AC, anchor cell. (B) Graded expression of the egl-17::cfp-lacZ reporter (arls92) (9). The events shown encompass approximately 6 hours.

Fig. 2. Functional assays for negative regulation of the EGFR-MAPK pathway in vulval development. The 10 potential lst genes identified biocomputationally were all tested in both assays. A positive result in either assay indicates that a candidate functions as a negative regulator of EGFR-MAPK activity. (A) Multivulva (Muv) phenotype in a gap-1(0) genetic background. gap-1(0) and other negative regulators alone do not perturb vulval induction but in combination synergize to cause ectopic induction of P3.p, P4.p, and P8.p (12). Photomicrographs show an invagination due to ectopic induction for lst-2(RNAi). The bar graphs show the number of Muv animals/total in a gap-1(0) background for each gene examined in this assay. The dpy-23(e840) null allele (28) was used for all experiments. RNA interference (RNAi) was performed as in (29). (B) Ectopic expression of egl-17::cfp-lacZ in the VPC daughters (=p.n.p stage). Photomicrographs show ectopic expression when lst-2 activity is reduced. The bar graphs indicate the number of animals with ectopic expression/total for each gene analyzed. White bars denote controls and negative results in this assay. Wild-type was used as a control for conventional mutations, and mock RNAi was used as a control for RNAi experiments. Black bars denote new candidate negative regulators identified in this study. Shaded bars denote mutations that reduce (−) or eliminate (0) the function of previously characterized negative regulators of EGFR-MAPK pathway used to validate this assay [gap-1(0), ga133; unc-101(−), sy108; lip-1(0), zh15; ark-1(−), sy247] (30). Note that we also identified ark-1 as a candidate lst gene.

To assay negative regulation of EGFR-MAPK activity directly in prospective 2° cells without the potentially confounding effect of a cell-fate transformation, we developed a new functional assay (Fig. 2B) based on the observation that egl-17 expression normally becomes restricted to the daughters of P6.p (Fig. 1B). We reasoned that, if the loss of egl-17::cfp-lacZ expression in presumptive 2° cells reflects negative regulation of the EGFR-MAPK pathway by lst gene activity, then depletion of lst gene activity would cause egl-17::cfp-lacZ expression to persist. We tested this assumption by depleting the activity of known negative regulators such as gap-1(0) and observed persistent expression of CFP in the daughters of VPCs other than P6.p without perturbation of vulval development, i.e., without a cell-fate transformation. Using this assay, we found that reducing the activity of ark-1, dpy-23, lst-1, lst-2, lst-3, and lst-4 results in ectopic egl-17 reporter expression in P5.p and P7.p daughters, indicating that these genes negatively regulate EGFR-MAPK signaling in the presumptive 2° VPCs (Fig. 2B). We note that the strongly positive results for lst-1 and lst-3, as compared with the gap-1(0) ectopic induction assay, suggest that persistent egl-17::cfp-lacZ expression is a very sensitive assay.

Transcriptional reporters (15) for the new negative regulators display strong and dynamic expression in the VPCs consistent with...
transcriptional response to the lateral signal. There are two general patterns. For genes that display Pattern A (dpy-23, lst-3), expression is very faint in the VPCs prior to the mid-L3 stage, but then becomes strong in P5.p and P7.p, and persists in their daughters (Fig. 3A). This pattern suggests that expression of these genes is activated upon lateral signaling, an inference that was confirmed by mutation of the LBS cluster for dpy-23 (Fig. 3B).

For genes that display Pattern B (lst-1, lst-2, and lst-4), uniform high expression is initially evident in all six VPCs, but at the time of inductive signaling, expression forms a gradient that is the inverse of the egl-17 reporter expression pattern in response to inductive signal: Expression of Pattern B genes is low in P6.p, intermediate in P5.p and P7.p, and undiminished in P3.p, P4.p, and P8.p. Later, expression becomes strong again in P5.p and P7.p and their daughters, but remains low in P6.p and its daughters (Fig. 3A). Mutation of the LBS cluster in lst-1 does not affect the initial inverse gradient of expression but abrogates up-regulation in P5.p and P7.p (Fig. 3B). A simple model to explain Pattern B is that the graded inductive signal initially leads to graded transcriptional repression of these lst genes, perhaps mediated by lin-1 (16), but that lateral signaling subsequently restores full expression in P5.p and P7.p.

In sum, we have provided evidence that a spatially graded inductive signal has a detectable impact on VPCs other than P6.p. We have also shown that, in addition to the MAPK phosphatase lip-1 (14), LIN-12 activates the expression of many different negative regulators of the EGF-MAPK pathway in P5.p and P7.p, which seem likely to inhibit the activity of this pathway at different steps (13). The study of lip-1 raised the possibility that negative regulation of EGF-MAPK activity in presumptive 2° cells plays a role in their specification, but because lip-1(0) does not cause defects in an otherwise wild-type background (14), the contribution of this negative regulation to the final patterning remained an open question. However, our finding that there are multiple LIN-12 target genes that perform this function implies a high degree of redundancy in this regulatory process and suggests that the effect of the spatial gradient of inductive signal must be countered for proper VPC patterning. These considerations suggest the revised model for VPC specification shown in Fig. 4.

It may be important to squelch EGF-MAPK activity in the prospective 2° cells, because expression of 1° characteristics may have adverse effects. In P6.p, the presumptive 1° cell, the inductive signal leads to transcriptional activation of genes encoding the lateral signal (2). Inappropriate expression of ligands for LIN-12 in P5.p and P7.p might activate LIN-12 in the prospective 3° cells, confounding their correct cell-fate choice, or inhibit the ability of LIN-12 to be activated in P5.p and P7.p by ligand produced by P6.p (17, 18). In addition, in P6.p, activation of the EGF-MAPK pathway causes endocytosis-mediated down-regulation of LIN-12 (19); if inappropriate activation of the EGF-MAPK pathway in P5.p and P7.p were to reduce the level of surface LIN-12, it would be less available to be activated by the lateral signal.

Because the principles and pathways elucidated by studying C. elegans vulval development have been generally applicable to other organisms, we speculate that this highly redundant mode by which LIN-12/Notch antagonizes EGF-MAPK also operates in other situations. For example, there is evidence that Notch can function as either an oncogene or as a tumor suppressor, depending on the cellular context (20). Perhaps where Notch has been observed to act as a tumor suppressor, it may be instructive to examine the expression of orthologs of the lst genes and other negative regulators of the EGF-MAPK pathway.

References and Notes
2. N. Chen, I. Greenwald, unpublished observations.
8. A. S. Yoo et al., data not shown.
9. Materials and methods are available as supporting material on Science Online.
13. Four of the five new genes that behave as negative regulators of the EGF-MAPK pathway in the VPCs in functional assays have mammalian orthologs, defined as highest scoring “hits” in reciprocal Basic
Reactivation of the Paternal X Chromosome in Early Mouse Embryos

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It is generally accepted that paternally imprinted X inactivation occurs exclusively in extraembryonic lineages of mouse embryos, whereas cells of the embryo proper, derived from the inner cell mass (ICM), undergo only random X inactivation. Here we show that imprinted X inactivation, in fact, occurs in all cells of early embryos and that the paternal X is then selectively reactivated in cells allocated to the ICM. This contrasts with more differentiated cell types where X inactivation is highly stable and generally irreversible. Our observations illustrate that an important component of genome plasticity in early development is the capacity to reverse heritable gene silencing decisions.

X inactivation is the developmentally regulated silencing of one of the two X chromosomes in female mammals, providing the mechanism for dosage compensation of X-linked genes relative to XY males. In mouse embryos, there is imprinted X inactivation of the paternal X chromosome (Xp) in the extraembryonic trophectoderm (TE) and primitive endoderm (PE). In all other cells, X inactivation is random. Establishment of these patterns has been thought to occur in a lineage-specific manner, with imprinted X inactivation initiated only in TE and PE cells as they differentiate at the blastocyst stage. In contrast, ICM cells, which give rise to the embryo proper, have been thought to retain both X chromosomes in the active state until they differentiate and undergo random X inactivation during early postimplantation development (1–5). Paradoxically, Xist RNA, the cis-acting signal that initiates X inactivation, is expressed from Xp as early as the two-cell stage (6, 7). This has been rationalized by supposing that cells in the early embryo cannot respond to Xist RNA (6).

In a recent study, we have shown that recruitment of Eed-Ezh2 Polycomb-Group (PcG) complex to the inactive X (Xi) is required to establish trimethylation of histone H3 lysine-27 (H3-K27) in postimplantation embryos (8). In the course of analyzing localization of Eed-Ezh2 on Xi in preimplantation embryos, we found that PcG localization occurs in all cells of early- and mid-stage XX blastocysts, including those corresponding morphologically to the ICM (Fig. 1A). In contrast, localization to Xi was no longer detectable in the ICM region of late-stage blastocysts, despite high levels of the Eed-Ezh2 proteins in the nuclei (Fig. 1B). Scoring data demonstrate that only early-stage blastocysts have Eed-Ezh2 foci in 100% of cells (Fig. 1C). In dual staining experiments, Ezh2 was seen to colocalize with Eed at all stages analyzed and Eed-Ezh2 foci colocalized with Xist RNA domains (fig. S1, A to C).

Eed-Ezh2 localization to Xi in the ICM was confirmed using dual staining for Eed and the recently described homeodomain protein Nanog, expressed specifically in ICM cells (9, 10). Eed foci were detectable in all Nanog-positive cells in early- and mid-stage blastocysts (Fig. 1, D and E) but were progressively lost at later stages (Fig. 1F). Interestingly, loss of Eed foci in the ICM region of maturing blastocysts occurs specifically in Nanog-positive cells, representing precursors of the embryo proper, but does not occur in Nanog-negative cells, which represent the primitive endoderm lineage.

Our observations led us to consider that imprinted X inactivation may occur in all cells of early blastocysts and may then be selectively reversed in the ICM, establishing the ground state for subsequent random X inactivation. To determine whether early ICM cells exhibit other markers of X inactivation, we carried out dual labeling for Eed and specific modifications

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