import, the precursor was added at the second incubation step. For cross-linking, mitochondria containing accumulated ^{35}S -labelled preproteins were re-isolated through a sucrose cushion^{5,12}, washed and incubated with EGS. After quenching of the crosslinker with Tris buffer, the proteins were precipitated with trichloroacetic acid. The samples were either analysed by SDS-PAGE or lysed in SDS-containing buffer and immunoprecipitated in Triton X-100-containing buffer¹². Standard procedures were used for immunoblotting onto nitrocellulose. Bands were detected by the Enhanced Chemiluminescence System (Amersham). Membrane potential was assessed using DiSC₃(5) (Molecular Probes)^{15,16}.

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X-chromosome-counting mechanisms that determine nematode sex

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Sex is determined in *Caenorhabditis elegans* by an X-chromosome-counting mechanism that reliably distinguishes the twofold difference in X-chromosome dose between males (1X) and hermaphrodites (2X)^{1,2}. This small quantitative difference is translated into the 'on/off' response of the target gene, *xol-1*, a switch that specifies the male fate when active and the hermaphrodite fate when inactive³. Specific regions of X contain counted signal elements whose combined dose sets the activity of *xol-1* (ref. 4). Here we ascribe the dose effects of one region to a discrete, protein-encoding gene, *fox-1*. We demonstrate that the dose-sensitive signal elements on chromosome X control *xol-1* through two different molecular mechanisms. One involves the transcriptional repression of *xol-1* in XX animals. The other uses the putative RNA-binding protein encoded by *fox-1* to reduce the level of *xol-1* protein. These two mechanisms of repression act together to ensure the fidelity of the X-chromosome counting process.

The control of sex by the number of X chromosomes was the first genetic regulatory mechanism to be described⁵. In the eighty-one years since its discovery, the mechanism by which chromosomes are counted to determine sex has been established only for *Drosophila*⁶. In *C. elegans*, the *xol-1* gene interprets X-chromosome number^{3,4}. Thus, the question of how the worm counts its X chromosomes has been reduced to the problem of identifying the elements that control *xol-1* activity and the mechanism by which they do so. Sex-specific regulation of *xol-1* is reflected in the tenfold-higher level of *xol-1* transcripts in males than in hermaphrodites³. Setting *xol-1* expression is essential for viability as well as sex determination because *xol-1* also controls X-chromosome dosage compensation⁷. Dosage compensation equalizes X-chromosome expression between the sexes by halving the level of transcripts made from each X chromosome in hermaphrodites^{6,8}. Inappropriate activation of *xol-1* in XX animals prevents the reduction of X expression and thereby causes hermaphrodite-specific lethality³. Repression of *xol-1* expression in XO animals causes inappropriate reduction of X expression and hence male-specific lethality^{3,7}.

To understand how the X-chromosome signal elements regulate *xol-1*, we first investigated whether the sex-specific difference in *xol-1* transcript levels results from transcriptional regulation. The activity of a *lacZ* reporter gene under the transcriptional control of the *xol-1* promoter (Fig. 1g) was assayed in males and hermaphrodites. We observed that XO animals carrying an integrated array (*yIs33*) containing multiple copies of the *Pxol-1::lacZ* reporter transgene had high β -galactosidase activity (Fig. 1c) and that array-bearing XX animals had low activity (Fig. 1b), indicating that transcriptional regulation is central to the sex-specific expression of *xol-1*.

Our previous genetic analysis demonstrated that X-chromosome dose is communicated to *xol-1* by elements in at least three distinct chromosomal regions⁴ (Fig. 1a). Duplication of all three regions causes >99% XO-specific lethality⁴. This lethality is suppressed by constitutive expression of *xol-1*. Deletion of one copy of all three regions in XX animals causes lethality and severe dosage compensation defects that are suppressed by *xol-1* null mutations⁴. To understand how individual components of the signal regulate *xol-1*, we asked whether deletions that eliminate X signal elements affect

the transcription of the *Pxol-1::lacZ* reporter gene. XX embryos from hermaphrodites heterozygous for a deletion that removed the signal element(s) in region 1 (*yDf19*) activated transcription of the *Pxol-1::lacZ* reporter, indicating that region 1 mediates transcriptional control of *xol-1* (Fig. 1d). In contrast, XX embryos from hermaphrodites heterozygous for a deletion that eliminated the signal elements in regions 2 and 3 (*yDf20*) failed to activate the reporter (Fig. 1e). This difference in response cannot be explained by a difference in the relative contribution of the individual X signal elements to the total X signal, because a twofold increase in the dose of regions 2 and 3 causes greater XO-specific lethality (62%) than a

corresponding increase in the dose of region 1 (0%) (Table 1, and ref. 4). Because this lethality is suppressed by constitutive expression of *xol-1*, it is unlikely that the signal elements in regions 2 and 3 regulate a target other than *xol-1*⁴. Thus, the expression studies suggest that the X components of the signal control sex determination and dosage compensation using a combination of two mechanisms, only one of which involves transcriptional repression of *xol-1* in XX animals.

To identify specific X-chromosome signal elements and thereby elucidate the mechanism of X-chromosome counting, we conducted a genetic screen for X-linked suppressors of the XO-specific lethality caused by duplication of the three signal element regions within *mnDp66* and *yDp14* (Fig. 1a, and ref. 4). Based on previous experiments that showed synergism among these signal element regions⁴, we anticipated that loss-of-function mutations in an X signal element would suppress the male lethality by lowering the total dose of signal elements in the duplication-bearing XO animals. From 24,000 mutagenized X chromosomes, we identified two potential signal-element mutations, *y303* and *y304*, and one deficiency that removes region 1. Both *y303* and *y304* were genetically mapped to a small interval covered by *yDp14* that corresponds to region 3 (Fig. 1a).

The genetic properties of the *y303* and *y304* mutations indicate that they define X signal element(s) in region 3. Both *y303* and *y304* are dose-dependent suppressors of the XO-specific lethality caused by duplications that increase the number of X signal elements. The extent of suppression is dependent on the number of wild-type copies of the element identified by *y303* and *y304*. Less than 1% of duplication-bearing XO animals are viable if they have twice the male dose of the elements in regions 1–3 (either one copy of *yDp13* or *mnDp66/yDp14*) (Table 1). In contrast, 100% of these duplication-bearing XO animals are viable if they also carry either *y303* or *y304* (Table 1). Suppression is less effective if more signal elements in region 3 are duplicated. Only 13% of *y303* males and 8.4% of *y304* males are viable if they have two copies of *yDp14*, a dose of signal elements in regions 2 and 3 (Table 1) that would otherwise kill 100% of XO animals.

In addition, *y303* and *y304* cause a synergistic increase in the dosage compensation defects of XX animals that carry deletions of other X signal elements. All XX animals homozygous for either *y303* or *y304* are viable and appear to be completely wild-type (Fig. 2a,b),

Figure 1 *xol-1* is transcriptionally regulated. **a**, Genetic map of the X chromosome left end shows three dose-sensitive regions (boxed) that contain counted components of the sex-determination signal. Regional boundaries are defined by deficiency (single line) and duplication (double line) endpoints⁴. **b–f**, Photomicrographs of embryo-laden midsections of hermaphrodites histochemically stained for β -galactosidase activity. Each hermaphrodite carries *yls33*, an integrated array with multiple copies of the *Pxol-1::lacZ* transcriptional reporter gene (**g**). This reporter includes 2.8 kb of *xol-1* genomic 5' regulatory sequences that direct *lacZ* expression. Comparison of β -galactosidase activity in XX embryos (**b**) with that in XO embryos (**c**) shows the transcriptional regulation of *xol-1*. The number of embryos showing intense β -galactosidase expression corresponds to the number of males expected from *him-5* mothers, namely 33% instead of the normal 0.2%. **d**, XX embryos deficient for region 1 (*yDf19*) have high β -galactosidase activity, indicating that signal elements in this region direct the transcriptional repression of *xol-1*. XX embryos deficient for either *fox-1* (**f**) or elements in regions 2 and 3 (*yDf20*) (**e**) have low β -galactosidase activity, suggesting that these signal elements are not transcriptional regulators of *xol-1*.

Table 1 *y303* and *y304* suppress XO-specific lethality caused by duplication of X-chromosome signal elements

Signal elements affected*	Genotype	Male viability (%)	n†
Dp(1) and Dp(2,3)‡	<i>mnDp66/yDp14; +</i>	1	660
Dp(1) and Dp(2,3) and <i>y303</i> ‡	<i>mnDp66/yDp14; y303</i>	109	1,031
Dp(1) and Dp(2,3) and <i>y304</i> ‡	<i>mnDp66/yDp14; y304</i>	96	1,450
Dp(1,2,3)\$	<i>+; yDp13</i>	0.4	266
Dp(1,2,3) and <i>y303</i> \$	<i>y303; yDp13</i>	90	1,001
Dp(1,2,3) and <i>y304</i> \$	<i>y304; yDp13</i>	94	747
Dp(2,3) and Dp(2,3)	<i>yDp14/yDp14; +</i>	0	>1,000
Dp(2,3) and Dp(2,3) and <i>y303</i>	<i>yDp14/yDp14; y303</i>	13	507
Dp(2,3) and Dp(2,3) and <i>y304</i>	<i>yDp14/yDp14; y304</i>	8.4	745
Dp(2,3)¶	<i>yDp14/+</i>	38	814
Dp(1)¶	<i>mnDp66/+</i>	102	1,874

* Dps are X-chromosome duplications containing one or more of the three signal element regions (Fig. 1a), indicated in parentheses. *yDp13* is a free duplication; *yDp14* and *mnDp66* are attached to chromosome I.

† n, Total number of animals counted.

‡ *yDp14/+*; *him-8*; *unc-2* males were crossed with hermaphrodites of genotype *mnDp66*; *unc-1 dpy-3* or *mnDp66*; *unc-1 dpy-3 y303* or *mnDp66*; *unc-1 dpy-3 y304*, and the per cent male viability was calculated as (total non-Dpy non-Unc males/one-half the total non-Dpy non-Unc hermaphrodites) $\times$ 100.

\$ *meDf6*; *yDp13* males were crossed with hermaphrodites of genotype *dpy-3 unc-2* or *dpy-3 y303 unc-2* or *dpy-3 y304 unc-2*, and the per cent male viability was calculated as (total non-Dpy non-Unc males/total non-Dpy non-Unc hermaphrodites) $\times$ 100.

|| Total live self progeny were counted from several individual hermaphrodites of genotype *yDp14/yDp14*; *him-8*; *unc-2* or *yDp14/yDp14*; *him-8*; *dpy-3 y303 unc-2* or *yDp14/yDp14*; *him-8*; *dpy-3 y304 unc-2*. Per cent male viability was calculated as (total males)/(0.37 $\times$ total expected progeny). Total expected progeny was calculated as (total hermaphrodites/0.63). These calculations are based on the fact that 37% of the self progeny of *him-8(e1489)* hermaphrodites are XO (ref. 14).

¶ Data from ref. 4.

as are XX animals heterozygous for *meDf6*, a deletion that removes the signal elements in regions 1 and 2. However, the combination of lesions in the *y303/meDf6* trans-heterozygous XX animals causes pronounced dosage-compensation defects (Fig. 2c). These dosage-compensation defects are comparable to those caused by deletions that remove all three signal element regions, either *yDf17* or the heterozygous combination of *yDf20/meDf5* (Fig. 2d). This genetic analysis reveals an important role for the locus defined by *y303* in counting X chromosomes and suggests that this locus can account for most, if not all of the X signal activity in region 3.

y303 and *y304* map to a region of X previously identified as causing XO-specific lethality and feminization when present in multiple copies⁹. The genetic locus responsible for the male lethal activity was not defined, but the activity was named *fox-1* (for 'feminizing locus on X') and was narrowed to a 30-kilobase (kb) segment of DNA⁹. A 1.8-kb embryonically expressed complementary DNA from within this segment was predicted to encode a 415-amino-acid protein with an RNP motif⁹, suggesting that the protein binds to RNA¹⁰. DNA sequence analysis of this RNP gene from our mutant strains revealed that the *y303* mutation causes an ochre translational stop at codon 37 and is therefore likely to cause complete loss of function (Fig. 2e). The *y304* allele causes a proline-to-serine change in the RNP motif at amino acid 161 of the same protein (Fig. 2e). This proline is predicted to be in a small loop region, between the β 1 sheet and the α 1 helix, and is conserved among many of the RNP proteins. This molecular information, together with our genetic analysis of *y303* and *y304*, establishes that the RNP gene, which we designate *fox-1*, is a *bona fide* X-chromosome sex-determination signal element. Moreover, because *y303* XX and XO animals are fully viable and fertile, and *y303* is probably a null allele, *fox-1* appears not to be an essential gene.

To learn how the FOX-1 protein functions in the X-chromosome counting process, polyclonal antibodies were raised against the entire 415-amino-acid protein and were used to determine the protein's location in wild-type and *fox-1*-mutant embryos. We expected a signal element to be expressed by the 28-cell stage and through gastrulation (400-cell stage) because *xol-1* must be active during that early period to trigger male development and must be repressed during that period to permit hermaphrodite development³. We observed very early nuclear staining in the cells of wild-type XX embryos beginning at the 8–16 cell stage of development; the staining intensity peaked by the 100-cell stage and was absent by the end of embryonic cell proliferation around the 550-cell stage (Fig. 2f). XX embryos with the *fox-1(y303)* nonsense mutation lacked staining, establishing the specificity of the antibody and confirming the classification of *fox-1(y303)* as a null allele (Fig. 2f). The staining results indicate that FOX-1 is present during an appropriate window of time to act as a component of the X signal and to downregulate *xol-1* in hermaphrodites.

fox-1 does not seem to regulate the transcription of *xol-1*. Transcriptional repression of *xol-1*, as monitored by the *xol-1::lacZ* transcriptional fusion (*yIs33*), was robust in XX embryos lacking *fox-1* (Fig. 1f), as we had found for XX embryos lacking the elements in regions 2 and 3 (Fig. 1e). To demonstrate that FOX-1 nonetheless acts on *xol-1* and to address the mechanism of repression, we used a different type of *xol-1* reporter gene, one that contained all the *xol-1* genomic sequences. In these experiments, the coding region of the green fluorescent protein (GFP) was fused in-frame to the first ATG codon of *xol-1*, creating a fully bifunctional GFP::XOL-1 fusion protein (Fig. 3d). Eight independent extrachromosomal arrays carrying multiple copies of this *gfp::xol-1* transgene were established in *xol-1* mutant strains. In each case, the array suppressed the XO-specific

Figure 2 The putative RNA binding protein, FOX-1, is a signal element. **a–d**, Bright-field photomicrographs comparing wild-type XX animals (**a**) to XX animals with X chromosome signal element mutations. **b**, *fox-1* mutants appear wild type. **c**, Loss of one copy of *fox-1* (+) and one copy of elements in regions 1 and 2 causes synergistic dosage compensation defects (dumpy and egg-laying-defective phenotypes) similar to those caused by loss of one copy of signal elements in

regions 1–3 (**d**). **e**, Signal element mutations *y303* and *y304* cause changes in the putative RNA binding protein, FOX-1. **f**, Confocal images of wild-type and *fox-1(y303)* embryos co-stained with FOX-1 antibody (green) and the DNA intercalating dye, propidium iodide (PI), (red). FOX-1 is nuclear localized in wild-type embryos and absent in *y303* embryos.

lethality caused by the *xol-1* mutation, indicating that the fusion protein has XOL-1 activity. All the arrays expressed high levels of GFP in XO (Fig. 3b) but not XX (Fig. 3a) animals. Moreover, none of these arrays caused XX-specific lethality or dosage compensation defects. Thus, these *gfp::xol-1* reporter arrays showed proper sex-specific regulation. They were crossed into strains that produce high levels of FOX-1 from an integrated array (*yls44*) containing multiple copies of the *fox-1* gene. The level of FOX-1 produced by *yls44* causes complete XO-specific lethality in otherwise wild-type strains. In *yls44* strains with the *gfp::xol-1* arrays, no GFP expression was detected (Fig. 3c) and all the XO animals died. These results indicate that high doses of FOX-1 drastically reduce the levels of both the GFP::XOL-1 protein and the endogenous XOL-1 protein, revealing that FOX-1 acts through *xol-1*.

In contrast to the eight *gfp::xol-1* arrays just described, which caused no XX-specific lethality, two other *gfp::xol-1* arrays expressed GFP::XOL-1 at a level that caused complete XX-specific lethality in strains with or without excess FOX-1. These two arrays also suppressed the male-lethal effect of excess FOX-1, suggesting that the FOX-1 activity had been titrated by *xol-1* sequences or protein. Such an interpretation is consistent with the fact that *xol-1* acts downstream of *fox-1*, and it suggests the possibility that *xol-1* may be the direct target of FOX-1.

Additional evidence for a post-transcriptional role for FOX-1 was provided by a different *xol-1* reporter construct in which the *gfp* coding sequences were fused in frame to a portion of the *xol-1* gene containing the promoter region, the 5'UTR, the first two introns and the first 89 codons (Fig. 3h). This fusion, called *Pxol-1::gfp*, does not provide *xol-1*(+) activity. Six independent extrachromosomal arrays carrying multiple copies of this reporter gene cause strong GFP staining in XO (Fig. 3f) but not XX (Fig. 3e) animals,

mimicking the sex-specific expression of *xol-1*. Excess FOX-1 produced from *yls44* failed to repress this translational *Pxol-1::gfp* fusion, indicating that the *fox-1* target sequences are not present in this construct (Fig. 3g). These results, together with the nuclear localization and the similarity of FOX-1 to RNA-binding proteins, suggest that FOX-1 regulates *xol-1* at a post-transcriptional step, such as splicing, stability, transport or translation of the *xol-1* RNA.

Our results reveal the general molecular strategy by which *C. elegans* amplifies the twofold difference in X chromosome dose to set sexual fate through effects on *xol-1* (Fig. 3i). One class of X signal elements represses the transcription of *xol-1* in XX animals. A second class, including the RNA-binding protein FOX-1, further reduces *xol-1* activity in XX animals through a post-transcriptional mechanism. The absence of obvious defects in XX animals lacking *fox-1* activity does not mean that the post-transcriptional mechanism has little significance in the counting process, because elements in region 2 that participate in this aspect of regulation remain in these mutants. Indeed, a female dose of the combined post-transcriptional elements in regions 2 and 3 reduces the viability of XO animals by half. The significance of the post-transcriptional mechanism is further demonstrated by the synergistic effect of mutations that partially inactivate both mechanisms in XX animals. Hermaphrodites with a male dose of region 1 (transcriptional) or a male dose of regions 2 and 3 (post-transcriptional) are wild-type, but hermaphrodites with a male dose of all three regions exhibit severe dosage compensation defects and death. Thus, the two mechanisms of repression act together to ensure the fidelity of the X chromosome counting process.

We can compare the X-chromosome counting process in *C. elegans* with that in *Drosophila melanogaster*. The fruitfly uses the XX dose of its signal elements to activate transcription of the target

Figure 3 *fox-1* represses *xol-1*. **a–c**, Fluorescence photomicrographs of embryo-laden midsections of hermaphrodites carrying *yEx(gfp::xol-1)*, an extrachromosomal array with multiple copies of the *gfp::xol-1* reporter gene in **d**. In this reporter, the GFP coding sequence was fused in frame to the first ATG of *xol-1*, thus creating a bifunctional GFP::XOL-1 fusion protein. The sex-specific expression of *yEx(gfp::xol-1)* is shown by the absence of GFP fluorescence in XX embryos (**a**), and the presence of GFP fluorescence in XO embryos (**b**). **c**, Excess FOX-1 provided from an integrated *fox-1*(+) array, *yls44*, represses embryonic GFP fluorescence, showing that FOX-1 acts through *xol-1*. **e–g**, Midsections of hermaphrodites carrying *yEx(Pxol-1::gfp)*, an extrachromosomal

array containing multiple copies of the *Pxol-1::gfp* translational reporter gene in **h**. In this reporter, the *gfp* coding region replaces *xol-1* sequences downstream of the 89th codon. **e, f**, *Pxol-1::gfp* is sex-specifically expressed. **g**, Excess FOX-1 fails to repress GFP fluorescence, indicating that *Pxol-1::gfp* lacks FOX-1 target sequences. **i**, A model for X chromosome counting by *xol-1*. In XX embryos, *xol-1* is inactivated in response to a hermaphrodite dose of X-chromosome signal elements. Complete repression of *xol-1* activity involves at least two concerted mechanisms, one acting transcriptionally and a second, mediated by the FOX-1 RNA-binding protein, acting post-transcriptionally. In XO embryos, *xol-1* remains active despite the male dose of X-chromosome signal elements.

switch gene, *Sex-lethal*, and thereby initiate female development⁶, whereas *C. elegans* represses expression of its target *xol-1* through transcriptional and post-transcriptional mechanisms. No evidence exists for post-transcriptional regulation operating in the initial X counting process of flies, although they do use a post-transcriptional mechanism to lock in the counting choice⁶. Mammals must also assess their X-chromosome number to achieve dosage compensation through X inactivation. Although the mechanism has not been determined, the evidence available¹¹ implies that it is likely to differ from the chromosome-counting mechanisms in both flies and worms. □

Methods

Genetic mapping. *y303* and *y304* were isolated using a previously described screen⁴. Of 948 embryos counted from *fox-1(y303)* XX hermaphrodites, all developed into fertile adults. *y303* was mapped between *lin-32* and *unc-2* by analysing Unc non-Dpy and Dpy non-Unc recombinant progeny from *dpy-3 + y303 unc-2/lin-32 + +* mothers. Of 21 Unc non-Dpy recombinants, 4 recombined between *dpy-3* and *lin-32*, 5 between *lin-32* and *y303*, and 12 between *y303* and *unc-2*. Of 6 Dpy non-Unc recombinants, 2 recombined between *lin-32* and *y303* and 4 between *y303* and *unc-2*. *y304* was similarly mapped. Of 16 Unc non-Dpy recombinants, 4 recombined between *dpy-3* and *lin-32*, 7 between *lin-32* and *y304*, and 5 between *y304* and *unc-2*. Of 17 Dpy non-Unc recombinants, 5 recombined between *lin-32* and *y303*, and 12 between *y304* and *unc-2*. *yDf19* is a γ ray-induced mutation that fails to complement *egl-17* and *unc-1* and does not cause X-chromosome non-disjunction. Of 976 self progeny from *yDf19/unc-1 dpy-3* mothers, none was male. *meDf6* removes *lin-32* but probably not *fox-1*. Fourteen dead embryos from *meDf6/dpy-3 unc-2* mothers amplified a control band and a *fox-1*-specific band in single-embryo polymerase chain reactions (PCRs). *yDf20* is a suppressor of the XO-specific lethality caused by two copies of *yDp14* (I. Carmi and B.J.M., unpublished results) and deletes *dpy-3*, *lin-32* and *fox-1*. Fifteen dead embryos from *yDf20/unc-1 dpy-3* mothers that amplified a control band failed to amplify a *fox-1*-specific band in single-embryo PCR. *yDf17* was isolated as a suppressor of the XO-specific lethality caused by *mnDp66/yDp14*. *yDf17* removes the left end of X, including *unc-2*, and *yDf17/+* XX animals have an Sdc phenotype similar to that of *yDf14/+* XX animals (ref. 4, and I. Carmi and B.J.M., unpublished results). For single-embryo PCRs¹², the primer pairs 5'-CCCATTGAGATTCAGAGACC and 5'-CGAGTGAACACGAGCTGTAG amplified an internal fragment of *fox-1* (ref. 9). The control primer pairs 5'-CTACTGTGCGACAATGTTGGAATCCTC and 5'-GGGATTTCTGCGATTGCAAGATG amplified a fragment of *sdc-3*.

DNA sequence analysis of *y303* and *y304*. The DNA changes caused by *y303* and *y304* were determined by DNA sequence analysis of cDNA generated by reverse-transcribed PCRs of RNA extracted from mutant (*dpy-3 y303* and *dpy-3 y304*) and parental (*mnDp66; unc-1 dpy-3*) strains. *fox-1* was amplified using the primers 5'-CCGCTCGAGATGCAAGCCCTGTACCAACT or 5'-CAGTCGGCGTTTGGAAATGGATCC with 5'-CGGGATCCCACTCAATACGG AGTAAATCG. The PCR products for each strain were cloned for three independent reactions and were sequenced from three clones. All three sets of sequences from the *y303* strain revealed only a C-to-T transition at nucleotide 109, and from the *y304* strain, a C-to-T transition at nucleotide 481.

***xol-1* reporter genes and transgenic arrays.** The *Pxol::lacZ* transcriptional fusion (pMN21) was made by insertion of the 2,779-bp *EcoRI* fragment from the *xol-1* promoter region into the *SmaI* site of pPD95.03, an intron-rich version of pPD16.01 (ref. 13) (from A. Fire). The *xol-1/lacZ* junction occurs 71 bp upstream of the first ATG in *xol-1*, thus eliminating *xol-1*'s normal 5'UTR, made by the insertion of an SL1 *trans*-spliced leader 13 nucleotides upstream of that ATG. *yIs33* was made by integrating an extrachromosomal array containing pRF4, a plasmid encoding a dominant *rol-6(su1006)* marker, and pMN21 coinjected at 100:40 $\mu\text{g ml}^{-1}$. The *gfp*-tagged *xol-1* genomic fusion (pMN45) (*gfp::xol-1*) was made by inserting a PCR fragment of *gfp* coding sequences (a S65T, I167T variant from Y. Jin) in frame at the first ATG of *xol-1*. The *yEx(gfp::xol-1)* extrachromosomal arrays contain p76-16B, a plasmid that encodes *unc-76(+)*, and pMN45 co-injected at 150:10 $\mu\text{g ml}^{-1}$. The *Pxol-1::gfp* translational fusion (pMN15) was made by replacing the 3,441-bp *SphI*-*ApaI* fragment from genomic *xol-1* sequences with the 1,931-bp *SphI*-*ApaI* *gfp*

fragment from the expression vector pPD95.67, which encodes the S65C variant of GFP (from A. Fire). The *yEx(Pxol-1::gfp)* extrachromosomal arrays contain p76-16B and pMN15 coinjected at 150:20 $\mu\text{g ml}^{-1}$. More than one thousand embryos of each appropriate genotype were scored in experiments using *xol-1* reporter constructs. The XO-specific expression of transgenic arrays carrying these reporter constructs was shown by the lack of embryonic activity in transgenic strains that produced only wild-type XX embryos and the abundant embryonic activity in transgenic lines that produced wild-type XO embryos because they carried a *him-5* mutation¹⁴. Excess *fox-1* was produced from an integrated array, *yIs44*, which causes XO-specific lethality and contains multiple copies of the *fox-1*-containing cosmid R04B3 and pRF4.

FOX-1 antibodies. Rabbit polyclonal antibodies were raised against a bacterially expressed HIS6::FOX-1 fusion protein that included the entire FOX-1 protein. The *fox-1* open reading frame was cloned into the expression vector pRSET-A (Invitrogen), and the HIS6::FOX-1 fusion protein was expressed and purified according to the procedures of Qiagen. Antibodies were affinity-purified using a fusion protein containing the entire FOX-1 protein and the maltose-binding protein (New England Biolabs). Embryos were stained as described¹⁵ using a 1:50 dilution of affinity-purified FOX-1 antibodies.

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correction

Structure of the adenylyl cyclase catalytic core

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In Fig. 3, the sequence in line 2 from 908 to 943 should read PGEVLVHMLNELFGKFDQIAKENEC...MRIKILGDC, and from 1,004 to 1,022 should be NVLCGVIGLQKWQYDVWSH. An extraneous 19 amino acids appeared at the start of the third line of the last block of sequence. This line should therefore start with AGGRA.... Also, the correct GenBank accession code for bovine type 1 adenylyl cyclase is M25579. □