The Primary Sex Determination Signal of *Caenorhabditis elegans*

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ABSTRACT

An X chromosome counting process determines sex in *Caenorhabditis elegans*. The dose of X chromosomes is translated into sexual fate by a set of X-linked genes that together control the activity of the sex-determination and dosage-compensation switch gene, xol-1. The double dose of X elements in XX animals represses xol-1 expression, promoting the hermaphrodite fate, while the single dose of X elements in XO animals permits high xol-1 expression, promoting the male fate. Previous work has revealed at least four signal elements that repress xol-1 expression at two levels, transcriptional and post-transcriptional. The two molecularly characterized elements include an RNA binding protein and a nuclear hormone receptor homolog. Here we explore the roles of the two mechanisms of xol-1 repression and further investigate how the combined dose of X signal elements ensures correct, sex-specific expression of xol-1. By studying the effects of increases and decreases in X signal element dose on male and hermaphrodite fate, we demonstrate that signal elements repress xol-1 cumulatively, such that full repression of xol-1 in XX animals results from the combined effect of individual elements. Complete transformation from the hermaphrodite to the male fate requires a decrease in the dose of all four elements, from two copies to one. We show that both mechanisms of xol-1 repression are essential and act synergistically to keep xol-1 levels low in XX animals. However, increasing repression by one mechanism can compensate for loss of the other, demonstrating that each mechanism can exert significant xol-1 repression on its own. Finally, we present evidence suggesting that xol-1 activity can be set at intermediate levels in response to an intermediate X signal.

Almost 50 years have passed since Nigon's original discovery that the primary sex determination signal of *Caenorhabditis elegans* is the X:A ratio, the ratio of X chromosomes to sets of autosomes (Nigon 1949a,b, 1951). A more quantitative understanding of the X:A signal came later with an analysis by Madl and Herman (1979). Using polyploid strains, they showed that animals with an X:A ratio of 0.75 or higher (3X:4A, 3X:3A, and 4X:4A) develop as hermaphrodites, while animals with a ratio of 0.67 or lower (2X:3A, 2X:4A, and 1X:2A) develop as males. Only now, however, are the molecular mechanisms by which this signal is translated into developmental fate becoming understood.

Because the only genetic difference between diploid males (XO) and hermaphrodites (XX) is the number of X chromosomes, a first step toward understanding how the primary signal determines sex in *C. elegans* is learning how the dose of X chromosomes is assessed. This question has been the subject of several recent studies (Akerib and Meyer 1994; Hodgkin et al. 1994; Nicoll et al. 1997; Carmi et al. 1998), which demonstrated that the dose of X chromosomes is transmitted by a relatively small set of X-linked genes, called X signal elements. The dose of these elements directly regulates the activity of the developmental switch gene, xol-1. In XX animals, the double dose of signal elements represses xol-1 expression and promotes the hermaphrodite fate, while in XO animals, the single dose of signal elements allows high xol-1 expression and promotes the male fate.

By controlling the activity of xol-1 (Rhind et al. 1995), signal elements control both sexual fate and dosage compensation (Meyer 1997), an essential process that equalizes X gene expression between the sexes. The function of xol-1 in XO animals is to repress the activity of hermaphrodite-specific sex-determination and dosage-compensation genes (sdc; Miller et al. 1988; Figure 1). In XX animals, sdc-2 acts with sdc-1 and sdc-3 to initiate the hermaphrodite modes of sex determination and dosage compensation (Nonet and Meyer 1991; Davis and Meyer 1997; Dawes et al. 1999). In XO animals, the high levels of xol-1 repress sdc-2, thus preventing activation of dosage compensation and promoting male sexual development.

To date, four regions of X have been identified that act in a dose-dependent manner consistent with their harboring signal elements (Figure 2; Akerib and Meyer 1994; Carmi et al. 1998). Changes in the dose of these regions cause reciprocal effects in males and hermaphrodites: increasing the dose of these regions in XO animals represses xol-1 and promotes hermaphrodite development, causing death because of inappropriate
activation of dosage compensation, while decreasing their dose in XX animals derepresses xol-1 and promotes male development, causing death because of failure to activate dosage compensation. Within these regions, two signal elements have been molecularly characterized. The signal element fox-1 maps to region 3 and encodes a putative RNA binding protein (Hodgkin et al. 1994; Nicoll et al. 1997; Skipper et al. 1999), while the signal element sex-1 defines region 4 and encodes a nuclear hormone receptor homolog (Carmi et al. 1998). As the identities of these elements imply, X signal elements use at least two different mechanisms to repress xol-1. sex-1 (region 4) and the element(s) in region 1 repress xol-1 at a transcriptional level, while fox-1 (region 3) and the element(s) in region 2 repress xol-1 at a post-transcriptional level. sex-1 is known to associate with the xol-1 promoter and therefore represses xol-1 transcription directly (Carmi et al. 1998).

These results led to the following model for xol-1 regulation by signal elements (Figure 3). In XX hermaphrodites, the two copies of sex-1 and region 1 repress xol-1 transcription, resulting in low transcript levels. Two copies of fox-1 and region 2 further repress xol-1 expression at a post-transcriptional level, resulting in overall low XOL-1 protein levels and the hermaphrodite fate, including activation of dosage compensation. In contrast, the single dose of signal elements in XO males does not effectively repress xol-1 either transcriptionally or post-transcriptionally, resulting in high XOL-1 levels and the male fate.

This model leaves many questions unanswered. How can a twofold difference in the dose of X signal elements lead to opposite activity states of xol-1 and hence different developmental fates? Why are two mechanisms of repression necessary? What is the contribution of individual elements to the signal and can one element substitute for another? And finally, what are the cellular consequences of perturbations in the X signal?

To address some of these questions and gain further insight into the process of X-chromosome counting, we undertook a genetic analysis of X signal elements in C. elegans. Our approach was to vary the dose of one or more signal elements and examine the consequences in males and hermaphrodites. Our results indicate that both the multigenic nature of the X signal and the use of two different mechanisms to repress xol-1 increase...
the fidelity of the signal, thereby allowing a twofold difference in the dose of regulators to specify two different sexual fates.

MATERIALS AND METHODS

Strains and general methods: C. elegans strains were maintained as described (Brenner 1974). Abbreviations are as follows: dpy (dumpy), egl (egg-laying defective), fox (feminizing locus on X), him (high incidence of males), lin (lineage), lon (long), rol (roller), sdc (sex determination and dosage compensation), sex (sex element on X), tra (sexual transformer), unc (uncoordinated), and xol (XO lethal). The following mutations and chromosomal aberrations were used in this study:

Linkage group (LG) II: tra-2(n1106), unc-4(e1420).
LG IV: him-8(e1489).
LG V: sdc-3(y129), unc-7(e111).
LG X: egl-17(e133), unc-1(e1598n1201), dpy-3(e27), lin-32(u282), fox-1(y303), unc-2(e55), unc-20(e122), lon-2(e678), unc-18(e11), dpy-6(e41), sex-1(gm41, y263), unc-115(nm490), sdc-2(y74), y93), unc-9(e101), unc-3(e151), sdc1(n485).

Duplications: mnDp66 (X;I) (Herman and Kari 1989); yDp14 (X;I), yDp13 (X;I) (Akerib and Meyer 1994); yDP2 (X;II) (Meneely et al. 1994).

Deficiencies: yDf13 X (Nicoll et al. 1997), mDf5 X and mDf6 X (Villeneuve 1994); yDf17 X.

Unreferenced mutations are described in Riddle et al. (1997) or in this article. All crosses and experiments were performed at 20°C, unless specified otherwise.

To perform viability counts, 1–2 L4 hermaphrodites were picked onto fresh plates and transferred to fresh plates every 12 hr until they stopped laying embryos. The number of embryos was determined, and 3–4 days later the number of surviving adults was determined. Viability was calculated as (number of counted adults) / (number of counted embryos).

Analysis of yDf17 and yDp20: yDf17 was isolated from a screen for suppressors of the male-specific lethality caused by the combination of duplications mnDp66 and yDp14. mnDp66/yDp14; him-8; unc-1 dpy-3 L4 XX hermaphrodites were mutagenized with EMS and surviving males were sought among their F2 progeny. To determine if the rescued males were XO animals or masculinized XX animals, we crossed males to unc4 XX hermaphrodites and looked for male cross progeny. XO males should produce XO male progeny in this cross, but XX Tra males would not unless the Tra were dominant. Both male and hermaphrodite (non-Unc) cross progeny were observed, consistent with the males being XO. Cloned cross progeny hermaphrodites also produced male progeny, suggesting that the suppressor mutation not only rescued XO animals but also caused a dominant Him phenotype. Because deficiencies of the left end of X remove the pairing region, causing X nondisjunction and production of males (Villeneuve 1994), we suspected that the suppressor mutation might be a deficiency. We therefore crossed suppressed males (mnDp66/ yDp14; him-8; yDf17) to eg-17 unc-1 unc-2 X hermaphrodites. This cross yielded only Unc hermaphrodite progeny, indicating that the suppressor was a deficiency that removed both unc-1 and unc-2. A subsequent cross to unc20 lon-2 X hermaphrodites revealed that the deficiency did not remove unc20. yDf20 was isolated in a similar screen for suppressors of the male-specific lethality caused by two copies of yDp14. In this screen yDp14; him-8; unc-2 XX hermaphrodites were mutagenized with EMS and males were sought among their F1 progeny. yDf20 was shown to be X-linked because a cross to yDp14; him-8; unc-1 dpy-3 XX hermaphrodites produced only patroclinous (yDp14; him-8; yDf20, wild type) but no matroclinous (yDp14; him-8, unc-1 dpy-3; Unc) males. yDf20 complemented both sdc1 and sdc2, both of which are X-linked and are expected to suppress the male lethality caused by increases in the signal. The suppressor chromosome could not be homozygosed, suggesting that yDf20 caused lethality. Furthermore, yDf20 failed to complement dpy-3 and lin-32, indicating that it was a deficiency.

SDC-2 and DPY-26 localization: Antibody staining was performed as previously described (Chuang et al. 1994), except that embryos were quick frozen in liquid nitrogen immediately after addition of fixative and then fixed for 20 min at room temperature after thawing.

sex-1 alleles: Unless specified otherwise, all experiments described in this article were performed with both sex-1(y263) and sex-1(gm41). Because sex-1(y263) is more likely to resemble the null phenotype of sex-1 (Carmi 1998), only the results obtained with sex-1(y263) are shown for most experiments.

RESULTS

Studying the function of X signal elements by changing their dose in hermaphrodites and males: Our goal was to study the contribution of each individual signal element and each mechanism of repression toward the regulation of xol-1, to understand better how a twofold difference in X signal element dose can lead to different sexual outcomes. Our initial approach was to examine how a change in the copy number of individual elements or combinations of elements affects hermaphrodite and male fates. In hermaphrodites, we used mutations and deficiencies of signal elements to examine the consequences of reducing signal element copy number to the male level, while in males we used signal element duplications to examine the consequences of increasing
the copy number of elements to the hermaphrodite level.

Previous experiments, using a variety of xol-1 reporter genes, demonstrated that changes in the dose of signal elements directly affect the level of xol-1 expression (Akerib and Meyer 1994; Nicoll et al. 1997; Carmi et al. 1998). Furthermore, these studies demonstrated that the changes in xol-1 levels lead to characteristic sex determination and dosage compensation phenotypes that are dependent on xol-1 function. In this study we used these phenotypes as a measure of the effect on xol-1 repression caused by changes in X signal element dose.

To facilitate our analysis, we isolated two deficiencies of X that remove different signal element regions. These deficiencies were obtained in screens for suppressors of the XO-specific lethality caused by duplications of signal elements. In a screen for suppressors of the XO-specific lethality caused by duplicating regions 1, 2, and 3 (Figure 2), we isolated a deficiency of all three regions, yDf17 (Figure 2 and material s and methods). A deficiency of regions 2 and 3, called yDf20, was obtained in a separate screen for suppressors of the XO-specific lethality caused by duplication of these two signal element regions (Figure 2 and material s and methods).

Signal elements act cumulatively to repress xol-1 in hermaphrodites: Our results indicate that full xol-1 repression in hermaphrodites results from the combined action of individual signal elements, consistent with the multigenic nature of the X signal. As described below, a reduction in the copy number of any one or two signal elements, from two copies to one, did not affect hermaphrodite fate or viability, but when the copy number of three or four elements was simultaneously halved, defects in sex determination and dosage compensation ensued (Table 1). Complete lethality was achieved only when the copy number of four signal elements was simultaneously reduced.

Our data show a correlation between the decrease in signal element copy number and the severity of sex determination and dosage compensation defects in XX animals (Table 1). Sex determination defects are manifested as transformation (Tra) toward the male fate, while dosage compensation defects result in Dpy and Egl phenotypes and lethality, caused by inappropriate levels of X gene expression. Previous studies have already demonstrated that these defects are specific to the dosage-compensation and sex-determination pathways because they can be fully suppressed by xol-1 null mutations (Akerib and Meyer 1994; Carmi et al. 1998). At the healthy end of the spectrum, hermaphrodites lacking one copy of region 1 (meDf5/ +), one copy of fox-1 (fox-1/ +), or one copy of sex-1 (sex-1/ +) appeared wild type. Similarly, in the majority of cases where one copy of each of two signal elements was deleted, hermaphrodites were also wild type. Hermaphrodites heterozygous for deletions of regions 1 and 2 or deletions of regions 2 and 3 (meDf6/ + and yDf20/ +, respectively) as well as hermaphrodites lacking one copy each of sex-1 and fox-1 (fox-1 +/ + sex-1) or region 1 and fox-1 (yDf19/fox-1) all appeared wild type. The only exception involved meDf5/ + sex-1 hermaphrodites, which lack one copy of region 1 and one copy of sex-1, and appear slightly Dpy. A twofold reduction in three different signal elements led to significantly more severe phenotypes. For example, hermaphrodites missing one copy of region 1, fox-1, and sex-1 (yDf19/fox-1 sex-1) ranged from wild type to Dpy and Egl, while hermaphrodites missing one copy of regions 1, 2, and 3 (yDf17/+) were Dpy, Egl, Tra, or dead. Possible reasons for the difference in phenotypes among hermaphrodites with a reduced dose of three different elements are discussed later. Finally, when four copies of signal elements were simultaneously deleted (yDf17/ + sex-1), all hermaphrodites died. The complete lethality is most likely specific to the decrease in the signal because xol-1 mutations were previously found to suppress fully all the defects caused by each of these mutations alone. These results demonstrate that a reduction in the copy number of individual signal elements causes partial derepression of xol-1 and that strong xol-1 derepression only occurs when the copy number of all four signal elements is simultaneously reduced.

The complete hermaphrodite lethality observed in the last experiment suggests that reducing the dose of each of four elements by one copy mimics the male state, inactivating dosage compensation. This experiment provides the first example of a complete transformation from the hermaphrodite to the male fate resulting from a change in signal element dose within the physiologically relevant range that separates diploid hermaphrodites from diploid males, a reduction from two copies to one.

Cumulative repression of xol-1 by increasing signal element dose in males: By analogy to the situation in hermaphrodites, increasing signal element copy number in males from one to two copies also acts cumulatively to increase xol-1 repression (Table 2A). We found that, with one exception, increasing the copy number of any one signal element had little or no effect on male viability, but that increases in the copy number of two or more elements caused significant male lethality. The sexual fate of the animals was not affected by the increase in signal element copy number, and we did not observe any feminization, a result that is discussed later. In our studies, an increase in the copy number of sex-1, provided by the duplication stDp2, or an increase in the copy number of region 1, provided by the duplication mnDp66 (Akerib and Meyer 1994), caused no XO lethality, but a previous study suggested that one extra copy of fox-1 reduced male viability to ~75% (Akerib and Meyer 1994). Increases in the copy number of two elements caused no lethality in the case of sex-1 and region 1 (107% viability; mnDp66/ +; stDp2/ +), but
TABLE 1

Reduced xol-1 repression in XX animals by loss of signal elements

<table>
<thead>
<tr>
<th>Signal elements or regions deleted</th>
<th>Total no. deleted</th>
<th>xol-1 repression mechanism(s) affected</th>
<th>Relevant genotype</th>
<th>XX phenotypeα (viability)</th>
<th>Strength of mutant phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Df(1) <strong>4</strong></td>
<td>1</td>
<td>T</td>
<td>yDf19/+ or mDf5/+ Wild type</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>sex-1 <strong>4</strong></td>
<td>1</td>
<td>T</td>
<td>sex-1/+          Wild type (100%)</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>fox-1</td>
<td>PT</td>
<td>fox-1/+</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Df(1), fox-1</td>
<td>2</td>
<td>PT, T</td>
<td>yDf19 +/+        Wild type</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Df(1), sex-1 <strong>4</strong></td>
<td>2</td>
<td>T, PT</td>
<td>mDf6/+           Wild type</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Df(2, 3)</td>
<td>2</td>
<td>PT, PT</td>
<td>yDf20/+          Wild type (99%)</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Df(1), sex-1 <strong>4</strong></td>
<td>2</td>
<td>T, T</td>
<td>mDf6 +/+ sex-1   Wild type to slightly Dpy -/+</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Df(1), sex-1 <strong>4</strong></td>
<td>3</td>
<td>T, T, T</td>
<td>yDf19 +/+        Wild type to Dpy and Egl +</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Df(1, 2, 3) <strong>4</strong></td>
<td>3</td>
<td>T, T, PT</td>
<td>yDf17/+          Dpy, Egl, Dead (57%)</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Df(1, 2), fox-1 <strong>4</strong></td>
<td>3</td>
<td>T, T, PT</td>
<td>mDf6 +/+ fox-1 sex-1 Wild type to slightly Dpy and Egl</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Df(2, 3), sex-1 <strong>4</strong></td>
<td>3</td>
<td>T, T, PT</td>
<td>yDf20 +/+ sex-1  Very Dpy, Egl, Tra, Dead (29%)</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Df(1, 2, 3), sex-1 <strong>4</strong></td>
<td>4</td>
<td>T, T, PT, T</td>
<td>yDf17 +/+ sex-1  Dead</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

α Numbers in parentheses correspond to the signal element regions diagrammed in Figure 2.

β T and PT refer to transcriptional and post-transcriptional repression of xol-1, respectively.

γ Because of the correlation between severity of phenotype and extent of viability, mutant combinations that do not result in an enhancement of phenotype likely do not affect the extent of viability.

δ Data from Akerib and Meyer (1994).

ε mmDp66/-; yDf19 XO males were mated with unc1 dpy-3 fox-1 and, as a control, with unc1 dpy-3 XX hermaphrodites. All progeny were wild type and there was no difference in phenotype between the Unc non-Dpy-3 (yDf19/unc1 dpy-3 fox-1 and yDf19/unc1 dpy-3) progeny hermaphrodites from the two crosses.

β Data from Carmi et al. (1996).

κ Non-Unc hermaphrodite progeny of sex-1 XO males and fox-1 unc-2 XX hermaphrodites were compared with a control cross to unc2 XX hermaphrodites. No difference in phenotype was observed between the +/+ sex-1/fox-1 unc2 + and the +/+ sex-1/unc2 hermaphrodites.

ι Actual genotype was mDf6/unc2 dpy-6. All hermaphrodites of this genotype are wild type.

λ A cross between yDp14/++; unc2 XO males and yDf20/unc1 dpy-3 XX hermaphrodites yielded 440 Unc-2 (yDf20/unc2) and 1336 non-Unc non-Dpy-3 (yDp14/++; yDf20/unc2, yDp14/++; unc1 dpy-3/unc2, and unc1 dpy-3/unc2) hermaphrodite cross progeny. The viability of the yDf20/unc2 hermaphrodites was calculated as [3 (number of Unc2 hermaphrodites) / (number of non-Unc non-Dpy-3 hermaphrodites)] = 100%. These yDf20/unc2 hermaphrodites were not Dpy or Egl.

μ Most hermaphrodite progeny from a cross between sex-1 XO males and mDf5 +/+ unc1 dpy-3 XX hermaphrodites were wild type, but a few were slightly Dpy. Because dpy-3 is semidominant, the Dpy phenotype could either result from dpy-3/+ or from an interaction between sex-1 and mDf5. To determine whether the Dpy animals were +/sex-1/mDf5 +/+ sex-1/unc1 dpy-3 +, eight hermaphrodites from each class were cloned and their progeny were examined. Two of eight wild-type and seven of eight Dpy hermaphrodites produced Unc Dpy progeny, suggesting that most sex-1/mDf5 animals are wild type and that the Dpy phenotype was caused, in most cases, by the semidominance of dpy-3.

ν Hermaphrodite progeny from a cross between yDp13; mDf6 XO males and unc2 sex-1 XX hermaphrodites were compared to a control cross with unc2 dpy-6 XX hermaphrodites. The control cross produced only wild-type hermaphrodites, while all the experimental cross produced some slightly Dpy, Egl hermaphrodites, suggesting a weak interaction between mDf6 and sex-1.

ω Both wild-type (non-Unc) and Dpy Egl (non-Unc) hermaphrodite progeny were produced from a cross between fox-1 sex-1 XO males and yDf19/unc1 dpy-3 XX hermaphrodites. Sixteen hermaphrodites of each phenotype were cloned and their progeny were examined. Hermaphrodites that did not produce Unc Dpy-3 progeny were inferred to be fox-1 sex-1/yDf19. Ten of 16 wild-type hermaphrodites produced Unc Dpy-3 progeny, 5 did not, and 1 was sterile. Five of 16 Dpy Egl hermaphrodites produced Unc Dpy-3 progeny. These results suggested that the phenotype of fox-1 sex-1/yDf19 was variable from wild type to Dpy and Egl, but that some of the Dpy progeny resulted from the semidominance of dpy-3.

ρ Self progeny from yDf17/unc2 XX hermaphrodites included 494 Unc (unc2) and 567 Unc Dpy (yDf17/unc2) hermaphrodites. Because unc2 homozygotes should be fully viable and yDf17 homozygotes are dead, the viability of yDf17/unc2 hermaphrodites was calculated relative to unc2 progeny as (number of Unc Dpy hermaphrodites) / (number of Unc hermaphrodites) × 100%.

τ A cross between fox-1 XO males and mDf6 +/+ unc1 lon-2 XX hermaphrodites yielded both wild-type and Dpy Egl (non-Unc) hermaphrodite progeny. All Dpy Egl hermaphrodites produced Dpy Egl progeny and no Unc Lon progeny. The viability of these hermaphrodites was not determined. However, the severity of the phenotypes is comparable to that observed with yDf17 +/. Based on this observation, it has been previously argued that fox-1 represents all the signal activity found in region 3.

υ The progeny of sex-1 XO males and yDf20 +/+ unc1 dpy-6 XX hermaphrodites included wild-type hermaphrodites and a few very Dpy, very Tra, and sick hermaphrodites. Because +/+ sex-1/unc2 dpy-6 + hermaphrodites are wild type, the Dpy Tra animals were inferred to be sex-1/yDf20. To determine the viability of yDf20/sex-1 animals, yDp14/++; unc1 sex-1(y263) XO males were mated with yDf20/unc1 dpy-3 XX hermaphrodites producing 108 Unc-2 (yDf20/unc2 sex-1) and 1134 non-Unc non-Dpy-3 (yDp14/++; yDf20/unc2 sex-1, yDp14/++; unc1 dpy-3/unc2 sex-1, and unc1 dpy-3/unc2 sex-1) hermaphrodite cross progeny. The viability of the yDf20/unc2 sex-1 hermaphrodites was calculated as [3 (number of Unc2 hermaphrodites) / (number of non-Unc non-Dpy-3 hermaphrodites)] = 100%.

ω sex-1 XO males were mated with yDf17/unc2 dpy-6 XX hermaphrodites. The non-Unc progeny included wild-type hermaphrodites and a few very Dpy, very sick, Tra animals. Because sex-1/unc2 dpy-6 is wild type and yDf17 + is already Dpy, Egl, and Tra on its own, these results suggested that most transheterozygote (sex-1/yDf17) animals are dead.
**TABLE 2**

Effect of increased signal element dose in males

### A. Cumulative repression of xol-1 in XO animals by increased dose of X signal elements

<table>
<thead>
<tr>
<th>Regions duplicated</th>
<th>Total no. duplicated</th>
<th>xol-1 repression mechanism(s) affected</th>
<th>Relevant genotype</th>
<th>XO viability (%)</th>
<th>No. males observed/ no. expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dp(sex-1)</td>
<td>1</td>
<td>T</td>
<td>stDp2/ +</td>
<td>~100i</td>
<td>—</td>
</tr>
<tr>
<td>Dp(1)</td>
<td>1</td>
<td>T</td>
<td>mnDp66/ +</td>
<td>94i</td>
<td>462/ 489</td>
</tr>
<tr>
<td>Dp(3)</td>
<td>1</td>
<td>PT</td>
<td>mnDp6/ +; yDp13</td>
<td>75i</td>
<td>—</td>
</tr>
<tr>
<td>Dp(sex-1), Dp(1)</td>
<td>2</td>
<td>T, T</td>
<td>mnDp66/ +; stDp2/ +</td>
<td>107i</td>
<td>492/ 460</td>
</tr>
<tr>
<td>Dp(2,3)</td>
<td>2</td>
<td>PT, PT</td>
<td>yDp14/ +</td>
<td>50i</td>
<td>168/ 344</td>
</tr>
<tr>
<td>Dp(1,2,3)</td>
<td>3</td>
<td>T, PT, PT</td>
<td>yDp13</td>
<td>6</td>
<td>13/ 227</td>
</tr>
<tr>
<td>Dp(sex-1), Dp(2,3)</td>
<td>3</td>
<td>T, PT, PT</td>
<td>yDp14/ +; stDp2/ +</td>
<td>13i</td>
<td>56/ 441</td>
</tr>
</tbody>
</table>

### B. Male lethality is due specifically to the increase in signal element dose

<table>
<thead>
<tr>
<th>Regions duplicated</th>
<th>Total no. duplicated</th>
<th>xol-1 repression mechanism(s) affected</th>
<th>Suppressor mutation</th>
<th>Relevant genotype</th>
<th>XO viability (%)</th>
<th>No. males observed/ no. expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dp(1,2,3)</td>
<td>3</td>
<td>T, PT, PT</td>
<td>sex-1</td>
<td>yDp13; sex-1</td>
<td>74</td>
<td>107/ 145</td>
</tr>
<tr>
<td>Dp(sex-1), Dp(2,3)</td>
<td>3</td>
<td>T, PT, PT</td>
<td>sdc-2</td>
<td>yDp14/ +; stDp2/ +; sdc-2</td>
<td>106</td>
<td>336/ 317</td>
</tr>
<tr>
<td>Dp(sex-1), Dp(2,3)</td>
<td>3</td>
<td>T, PT, PT</td>
<td>sex-1</td>
<td>yDp14/ +; stDp2/ +; sex-1</td>
<td>71</td>
<td>210/ 294</td>
</tr>
</tbody>
</table>

---

* Numbers in parentheses correspond to the signal element regions shown in Figure 2.
* T and PT refer to transcriptional and post-transcriptional repression of xol-1, respectively.
* stDp2 is attached to chromosome II, mnDp66 and yDp14 are attached to chromosome I, and yDp13 is a free duplication, present in one copy in the indicated strains.
* The viability of stDp2/ + XO males was deduced from experiments in which stDp2/ +; dpy-6 XO males were mated with dpy-6 or unc-2 dpy-6 XX hermaphrodites, and the numbers of hermaphrodite and male progeny with and without the duplication were counted. The number of hermaphrodites is expected to equal the number of males, but in all cases the number of duplication-bearing males was greater than the number of duplication-bearing hermaphrodites (the ratio of males to hermaphrodites was 341:271 and 190:112, respectively, for the two crosses). Because other experiments suggest that one copy of stDp2 causes no X-specific lethality, it appears that stDp2 segregates away from the X in the father, making it impossible to calculate male viability precisely.
* The ratio of Dpy (mnDp66/ +; unc-1 dpy-6) to Unc Dpy (unc-1 dpy-6) male progeny from a cross between mnDp66/ +; unc-1 XO males and unc-1 dpy-6 XX hermaphrodites is shown. The viability of mnDp66/ + males was calculated relative to that of unc-1 dpy-6 males, because equal numbers are expected for each class.
* Akerib and Meyer (1994).
* The ratio of wild-type (mnDp66/ +; stDp2/ +; unc-1 dpy-6) to Unc (+/ +; stDp2/ +; unc-1 dpy-6) male progeny produced by a cross between mnDp66/ +; unc-1 XO males and stDp2; unc-1 dpy-6 XX hermaphrodites is shown. The viability of mnDp66/ +; stDp2/ + was thus calculated relative to that of the stDp2/ + males generated in the same cross.
* The ratio of Dpy (yDp14/ +; unc-2 dpy-6) to Unc Dpy (unc-2 dpy-6) male progeny from a cross between yDp14/ +; unc-2 XO males and unc-2 dpy-6 XX hermaphrodites is shown. Because an equal number of males is expected in each class, the viability of yDp14/ + males was calculated relative to the viability of unc-2 dpy-6 males generated in the same cross.
* The ratio of wild-type male to wild-type hermaphrodite progeny produced by a cross between yDp13; meDp6 XO males and unc-1 lin-32 or unc-1 sex-1 XX hermaphrodites is shown. The viability of males was calculated relative to the number of hermaphrodites generated in the same cross.
* yDp14/ +; unc-2 XO males were mated with stDp2; unc-2 m sup XX hermaphrodites, m sup were dpy-6 +, dpy-6 sdc-2(y93) or unc-18 sex-1(y263). The ratio of wild-type (yDp14/ +; stDp2/ +; unc-2 m sup) to Unc (+/ +; stDp2/ +; unc-2 m sup) male cross-progeny is shown. The viability of yDp14/ +; stDp2/ + males was calculated relative to that of the stDp2/ + males generated in the same cross.

caused a significant reduction in viability in the case of regions 2 and 3 (50% viability; yDp14/ +). A further large reduction in viability resulted from the simultaneous duplication of three signal elements. A duplication of regions 1, 2, and 3 (yDp13) reduced male viability to 6%, and the combination of a sex-1 duplication and a duplication of regions 2 and 3 (yDp14/ +; stDp2/ +) reduced male viability to 13%.

To ensure that the male lethality observed in the last two experiments was specific to an increase in the X signal and thus due to the inappropriate activation of dosage compensation rather than misregulation of other genes on the duplications, we tested whether the lethality could be suppressed by mutations that disable dosage compensation. The male-specific lethality caused by yDp13 has previously been shown to be suppressed by mutations in the dosage-compensation gene sdc-2 (Akerib and Meyer 1994), and we found that an sdc-2 mutation similarly suppressed the male lethality caused by duplication of sex-1 and regions 2 and 3 (Table
Possible maternal component of the X signal element(s) in region 2

<table>
<thead>
<tr>
<th>Signal elements or regions deleted in zygote</th>
<th>Relevant genotype scored</th>
<th>Relevant maternal genotype</th>
<th>XX phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Df(1,2), sex-1*</td>
<td>mdDf6/sex-1</td>
<td>sex-1</td>
<td>Most wild type, some slightly Dpy</td>
</tr>
<tr>
<td>Df(1,2), sex-1†</td>
<td>mdDf6/sex-1</td>
<td>mdDf6/sex-1</td>
<td>Most slightly Dpy, some Dpy and Egl</td>
</tr>
<tr>
<td>Df(1,2,3)</td>
<td>yDf17/+</td>
<td>+/+</td>
<td>Dpy, Egl</td>
</tr>
<tr>
<td>Df(1,2,3)‡</td>
<td>yDf17/+</td>
<td>yDf17/+</td>
<td>Tiny Dpy, Egl, dead</td>
</tr>
<tr>
<td>fox-1, Df(2,3)†</td>
<td>fox-1/yDf20</td>
<td>fox-1</td>
<td>Wild type, some slightly Dpy</td>
</tr>
<tr>
<td>fox-1, Df(2,3)†</td>
<td>fox-1(y303)/yDf20</td>
<td>yDf120/+</td>
<td>Wild type to Dpy and Egl</td>
</tr>
</tbody>
</table>

1 yDp13; mdDf6 XO males were mated with unc2 unc18 sex-1(y263) XX hermaphrodites and cross-progeny (non-Unc) hermaphrodites were cloned. Because mdDf6 causes X nondisjunction the genotype of the hermaphrodites can be inferred from the phenotype of male self-progeny: only hermaphrodites carrying yDp13 produce wild-type males (yDp13; mdDf6), while the hermaphrodites of interest (unc2 unc18 sex-1/mdDf6) produce only Unc males. Most sex-1/mdDf6 F1 hermaphrodites were wild type to slightly Dpy, while F2 hermaphrodites varied from wild type to very Dpy and Egl.

2 Unc non-Lon progeny from a cross between yDp13; yDf17 XO males and unc1 lon-2 XX hermaphrodites or Unc Dpy self-progeny from yDp14/++; him-8/++; yDf17/unc2. The viability of these hermaphrodites was not determined.

3 A cross between fox-1 XO males and yDf20/+; unc2 lon-2 XX hermaphrodites produced both wild-type (non-Unc) and Dpy (non-Unc) progeny. Sixteen hermaphrodites of each phenotype were individually cloned and their progeny were examined. Hermaphrodites that did not produce Unc Lon progeny were inferred to be fox-1/yDf20. Thirteen of 16 Dpy hermaphrodites produced wild-type and Dpy Egl progeny, 2 of 16 produced Unc Lon progeny, and 1 of 16 was sterile. Of the wild-type hermaphrodites, 1 was sterile and 15 produced Unc Lon progeny. These results suggested that fox-1/yDf20 hermaphrodites were mostly Dpy and Egl. However, when yDp14/++; yDf20 males were crossed with fox-1 unc2 lon-2 or fox-1 unc2 dpy-6, the phenotype of most Unc non-Lon or Unc non-Dpy hermaphrodite progeny was wild type, with a few slightly Dpy hermaphrodites.

Maternal dose of signal elements can influence the sexual fate of the zygote: By definition, signal elements must act zygotically to specify the sexual fate of the embryo. Yet at least one signal element, sex-1, was also found to have a strong maternal component: the viability of homoyzgous sex-1 hermaphrodites is dependent on the maternal genotype and is much lower when both mother and zygote are homozygous for a sex-1 mutation (Carmi et al. 1998). The viability of sex-1 homozygotes is 65% for m' z' sex-1 animals compared to 90% for m' m' animals, where m indicates the maternal and z the zygotic genotype of sex-1. Other mutant combinations (Table 3) imply the existence of a maternal component for other signal elements besides sex-1. Specifically, mutants with a reduced copy number of region 2 cause more severe dosage compensation and sex determination phenotypes if the mother also has a reduced copy number of region 2. While most mdDf6 +/+ sex-1 XX hermaphrodite progeny from sex-1 homozygous mothers are wild type, their own genetically identical hermaphrodite progeny vary from slightly Dpy to Dpy and Egl. Similarly, yDf17/+ hermaphrodite progeny from wild-type mothers are not as Dpy and Egl as yDf17/+ progeny from yDf17/+ mothers. And finally, yDf20/fox-1 hermaphrodite progeny from fox-1 mothers are at most slightly Dpy, while those coming from yDf20/+ mothers can be Dpy and Egl. In all cases where stronger phenotypes are observed, the mother has a reduced copy number of two or more elements and the common element is region 2, suggesting that the element(s) in region 2, like sex-1, has a maternal component.

We do not know the significance of the maternal component, but it seems most likely that it is specific to the sex determination process. At least for sex-1, both the maternal and zygotic phenotypes affect only XX animals and are fully suppressed by xol-1 mutations, indicating that they are very specific to sex determination and dosage compensation (Carmi et al. 1998). Perhaps the maternal component establishes a basal level of signal element product in the egg. This maternal level may not be high enough to repress xol-1 and prevent the male fate, but the addition of zygotic product from both X chromosomes of hermaphrodites then leads to full xol-1 repression. In this way the relevant dose, the "counted dose," is still zygotic.

Both transcriptional and post-transcriptional mechanisms are necessary for proper xol-1 repression in hermaphrodites: Signal elements act at two different levels,
transcriptional and post-transcriptional, to repress \textit{xol-1}, and our experiments provide the first direct evidence for the essential contribution of post-transcriptional repression. The importance of transcriptional repression was known previously because homozygous mutations in \textit{sex-1}, a transcriptional repressor of \textit{xol-1}, cause strong sex determination and dosage compensation defects in hermaphrodites, including significant lethality (Carmi et al. 1998). The contribution of the post-transcriptional repression mechanism by itself has been less clear, however, because homozygous mutations in the post-transcriptional regulator \textit{fox-1} cause no observable defects in hermaphrodites.

Region 2 also appears to participate in post-transcriptional regulation of \textit{xol-1}. Changes in the dose of region 2 strongly affect \textit{xol-1} regulation in combination with other signal element duplications and deficiencies. If region 2 regulated \textit{xol-1} transcriptionally, changes in its dose would affect the expression of \textit{xol-1::lacZ} or \textit{xol-1::gfp} transcriptional reporter genes, as do changes in the doses of regions 1 and 4. However, changes in the dose of region 2 have no effect on these reporter genes (Akerib and Meyer 1994; Nicoll et al. 1997). While it is possible that region 2 has a very weak transcriptional repressor of \textit{xol-1}, its robust synergistic effect with other signal elements makes it more likely that region 2, like region 3, has a strong post-transcriptional repressor of \textit{xol-1}.

To test the contribution of post-transcriptional repression, we examined the consequence of reducing the copy number of region 2 in a fox-1 mutant background. We found that \textit{yDp20/+} fox-1 hermaphrodites, which lack fox-1 and have a reduced copy number of region 2, ranged from wild type to Dpy Egl and had reduced viability (84%). This result demonstrates that disabling the post-transcriptional regulation by itself leads to measurable derepression of \textit{xol-1}. The post-transcriptional mechanism is therefore essential for keeping \textit{xol-1} levels low in hermaphrodites, and the two mechanisms of repression, transcriptional and post-transcriptional, are not redundant.

A reduction in the dose of elements involved in one mechanism of \textit{xol-1} repression can counteract an increase in the dose of elements involved in the other: If both mechanisms of \textit{xol-1} repression, transcriptional and post-transcriptional, are important and act as two independent pathways that control \textit{xol-1}, one prediction is that a decrease in repression at one level will counteract an increase in repression at the other level. We found that this prediction holds in both males and hermaphrodites.

In males, eliminating \textit{sex-1}, a transcriptional repressor, counteracted an increase in the copy number of post-transcriptional repressors in regions 2 and 3, while eliminating the post-transcriptional regulator \textit{fox-1} counteracted an increase in the copy number of \textit{sex-1} (Table 4A and Carmi et al. 1998). \textit{sex-1} mutations fully suppressed the male lethality caused by one extra copy of \textit{yDp14}. The viability of \textit{yDp14}-bearing males was 38% in an otherwise wild-type background, but increased to 98% in a \textit{sex-1} mutant background. Similarly, \textit{fox-1} mutations fully suppressed the male-specific lethality caused by two extra copies of \textit{sex-1} (\textit{stDp2/ stDp2}), raising their viability from 30 to 95%. These experiments indicate that reciprocal changes in the strength of transcriptional and post-transcriptional regulation counteract each other in males and provide further evidence for the importance of the two mechanisms.

In hermaphrodites, increasing the dose of post-transcriptional repressors weakly counteracted a loss of transcriptional repression by \textit{sex-1} (Table 4B). For example, two extra copies of regions 2 and 3 only partially suppressed the effects of \textit{sex-1} mutations in hermaphrodites. The viability of \textit{yDp14/yDp14}, \textit{sex-1} hermaphrodites was only slightly higher (74%) than the viability of \textit{sex-1} hermaphrodites without the duplication (65%). Although the difference in viability is statistically significant (\(P < 0.01\)), the rescue is very weak. Because two copies of \textit{yDp14} cause complete male lethality (Akerib and Meyer 1994), an increase in the dose of regions 2 and 3 has the ability to repress \textit{xol-1}. The failure to rescue \textit{sex-1} hermaphrodites suggests then that transcriptional repression is essential to keep \textit{xol-1} levels low in hermaphrodites and that even a double dose of the post-transcriptional elements in regions 2 and 3 cannot compensate for its loss. However, a large increase in the dose of post-transcriptional regulators should, in principle, lower \textit{xol-1} expression enough to rescue \textit{sex-1} hermaphrodites. This appears to be the case because overexpression of \textit{fox-1} from an extrachromosomal array (\textit{yIs44}; M. Nicoll and B. J. Meyer, unpublished observations) at least partially rescues \textit{sex-1} hermaphrodites. In the presence of the array, \textit{sex-1} hermaphrodites appeared less Dpy and were not masculinized. These results are consistent with the two levels of repression acting independently to regulate \textit{xol-1} expression.

Synergy between transcriptional and post-transcriptional regulators shows the importance of the two mechanisms of \textit{xol-1} repression: A second prediction arising from the existence of two independent repression mechanisms is that a change in the dose of elements affecting both levels of repression should lead to a greater effect on \textit{xol-1} expression than a change affecting only one level. In this section we present evidence for the synergistic action of the two mechanisms of repression. In males, increasing the dose of both transcriptional and post-transcriptional regulators appears to cause synergistic lethality (Table 2A). For instance, although one extra copy of the transcriptional regulator in region 1 (\textit{mnDp66/+}) causes no male lethality and one extra copy of the post-transcriptional regulators in regions 2 and 3 (\textit{yDp14/+}) reduces viability to 50%, a duplication of all three regions (\textit{yDp13}) reduces male
viability to 6%. Similarly, the reduction in viability caused by duplicating the transcriptional repressor sex-1 alone (stDp2/++; 100% viability) or yDp14 alone (regions 2 and 3; 50% viability) is much smaller than the reduction caused by these two duplications together (yDp14/+; stDp2/++; 13% viability; Table 2A). The combined effects of the different duplications are greater than just additive, suggesting that a hermaphrodite (2X) dose of both transcriptional and post-transcriptional regulators is much more effective in repressing xol-1 than a hermaphrodite dose of regulators involved in either mechanism alone.

Such synergy between repression mechanisms may also exist in hermaphrodites. One example, shown previously, is the complete inviability of fox-1 sex-1 double mutant hermaphrodites that lack one transcriptional (sex-1) and one post-transcriptional (fox-1) repressor (Carmi et al. 1998). The complete lethality caused by the double mutant combination is much greater than the lethality caused by either homozygous mutation alone: fox-1 homozygous hermaphrodites are fully viable, while sex-1 homozygotes are 65% viable. Because this example involves complete elimination (loss of two copies) of both signal elements, it is not directly relevant to the genetic difference between wild-type XX and XO animals.

Of greater relevance to sex determination in vivo, when the copy number of signal elements in hermaphrodites is only reduced by half (Table 1), the results are also consistent with stronger phenotypes resulting from disruption of the two mechanisms of repression. Of mutants having a reduced dose of three signal elements, strong phenotypes only result when the copy number of both regions 2 and 3 (fox-1) and one transcriptional repressor (sex-1 or region 1) are simultaneously reduced, as in yDf17/+; meDf6/+ + fox-1, or yDf20 +/+ + sex-1. Weaker phenotypes result when the doses of the two transcriptional repressors and only one post-transcriptional repressor (fox-1 or region 2) are simultaneously reduced. A feasible explanation for the difference in phenotypic strength among these mutants is that both transcriptional and post-transcriptional mechanisms of repression must be disabled to cause strong defects in hermaphrodites. It appears that a twofold reduction in the dose of either sex-1 or region 1 is sufficient to disrupt transcriptional repression, but that the doses of both fox-1 and region 2 need to be reduced by half to disable post-transcriptional repression. Region 2 and fox-1 may regulate xol-1 by two different post-transcriptional mechanisms or they may be partially redundant.

Combined, these data confirm that the two mechanisms of xol-1 repression are required and act as two

### TABLE 4

Both xol-1 repression mechanisms, transcriptional and post-transcriptional, are important and act independently

<table>
<thead>
<tr>
<th>Regions duplicated (repression mechanism)</th>
<th>Suppressor mutation (repression mechanism)</th>
<th>Relevant XO genotype</th>
<th>Observed/expected XO male viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dp(2,3) [PT]</td>
<td>—</td>
<td>yDp14/+</td>
<td>193/505</td>
</tr>
<tr>
<td>Dp(2,3) [PT]</td>
<td>sex-1 [T]</td>
<td>yDp14/+; sex-1</td>
<td>294/300</td>
</tr>
<tr>
<td>Dp(sex-1), Dp(sex-1) [T]</td>
<td>—</td>
<td>stDp2/stDp2</td>
<td>160/516</td>
</tr>
<tr>
<td>Dp(sex-1), Dp(sex-1) [T]</td>
<td>fox-1 [PT]</td>
<td>stDp2/stDp2; fox-1</td>
<td>725/761</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Signal element mutation (repression mechanism)</th>
<th>Signal element duplication (repression mechanism)</th>
<th>Relevant genotype</th>
<th>Adults/embryos XX viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sex-1 [T]</td>
<td>—</td>
<td>sex-1</td>
<td>748/1150</td>
</tr>
<tr>
<td>—</td>
<td>Dp(2,3), Dp(2,3) [PT]</td>
<td>yDp14/yDp14</td>
<td>2083/2151</td>
</tr>
<tr>
<td>sex-1 [T]</td>
<td>Dp(2,3), Dp(2,3) [PT]</td>
<td>yDp14/yDp14; sex-1</td>
<td>2866/3890</td>
</tr>
</tbody>
</table>

1 The numbers correspond to the signal element regions shown in Figure 2.
2 T and PT refer to transcriptional and post-transcriptional repression of xol-1, respectively.
3 mnDp66 and yDp14 are attached to chromosome I. stDp2 is attached to chromosome II.
4 The ratio of wild-type (yDp14/+; unc-2 or yDp14/+; unc-21 sex-1) to Unc (unc-2 or unc-2 sex-1) male progeny from a cross between yDp14/+; lin-32 fox-1 XO males and unc-2 or unc-2 sex-1 XX hermaphrodites is shown. The viability of yDp14/+ males was calculated relative to the viability of the Unc males generated in the same cross.
5 Carmi et al. (1998).
6 Actual genotype was yDp14; unc-2.
7 Actual genotype was yDp14; unc-2 sex-1.
8 The rescue is significant based on the χ² test with P < 0.01.
independent pathways to regulate xol-1, such that the strongest phenotypes are obtained when both pathways are simultaneously disrupted (in hermaphrodites) or simultaneously enhanced (in males). The two pathways therefore act synergistically to ensure full repression of xol-1 in XX animals.

**Region 1 is partially dependent on sex-1 for xol-1 repression:** The data discussed so far pertain to the two mechanisms of xol-1 repression, transcriptional and post-transcriptional. The question still remains whether signal elements of the same regulatory mechanism act dependently or independently to repress xol-1. In this section we analyze the relationship between the transcriptional regulators, sex-1 and the signal element(s) in region 1 (collectively referred to as region 1), showing that region 1 is partially dependent on sex-1. This result suggests that these elements might act in a single pathway, possibly as corepressors.

Based on the genetic properties of the signal, we considered three different general possibilities for the relationship between sex-1 and region 1: (1) the two regulators could act as independent repressors in two redundant pathways to repress xol-1; (2) they could act as independent but not redundant repressors; or (3) they could be dependent on each other (i.e., act together as corepressors) to achieve full xol-1 repression. Each of these possibilities entails specific predictions about the effects caused by changes in the dose of the two regions. As we discuss below, these predictions are only fulfilled for the third possibility.

**sex-1 and region 1 do not appear to act in redundant pathways.** If repression by the two elements were redundant, a deletion of either one would not be expected to affect xol-1 repression, but a deletion of both would be expected to cause strong synergistic effects. This prediction is contradicted by our data, because deletions of either sex-1 or region 1 lead to significant xol-1 derepression. sex-1 mutations cause strong dosage compensation and sex determination defects (Car mi et al. 1998), and either sex-1 mutations or a deletion of region 1 (Nicoll et al. 1997) derepresses a xol-1::lacZ transcriptional fusion, causing lacZ expression in XX animals. In wild-type animals, the xol-1::lacZ reporter gene reflects the normal sex-specific expression of xol-1, showing high levels of expression in XO and low levels of expression in XX embryos. Furthermore, reductions in the copy number of sex-1 and region 1 do not cause synergistic effects. A deletion of region 1 does not enhance the hermaphrodite phenotype caused by sex-1 mutations because yDF19 sex-1/ + sex-1 hermaphrodites are as Dpy and Egl as sex-1/ sex-1 hermaphrodites (not shown). These experiments suggest that region 1 and sex-1 do not act synergistically to repress xol-1 and therefore are unlikely to act in two redundant pathways.

**sex-1 and region 1 also do not appear to act completely independently because an increase in the dose of region 1 cannot counteract a decrease in the dose of sex-1. If region 1 acted independently of sex-1, increasing its dose should increase xol-1 repression even in the absence of sex-1. We found that two extra copies of region 1 (mnDp66/ mnDp66; Table 5) could not suppress the hermaphrodite phenotypes caused by sex-1 mutations. The viability of mnDp66/ mnDp66; sex-1 hermaphrodites was 30%, compared to 76% viability of mnDp66/ mnDp66 hermaphrodites. Because two extra copies of region 1 cause significant male lethality in an otherwise wild-type background (Table 5) and therefore can exert significant repression on xol-1, the failure to compensate for the loss of sex-1 suggests that region 1 requires sex-1(+ ) to achieve full xol-1 repression. Region 1 is therefore unlikely to be completely independent of sex-1.

Other data are consistent with region 1 being dependent on sex-1 and possibly acting as a corepressor with sex-1. We found that sex-1 mutations suppressed the male-specific lethality caused by the increased dose of region 1. sex-1 mutations increased the viability of mnDp66/ mnDp66 males from 29 to 56% (Table 5). The remaining male lethality in the sex-1 background is likely a result of non-sex-specific effects of mnDp66. Two extra copies of mnDp66 cause both male-specific and general lethality, reducing hermaphrodite viability to 76% (Table 5). Because the increased dose of region 1 kills males by improperly activating dosage compensation, sdc-2 mutations, which disrupt dosage compensation, can be used to separate the sex-specific from the non-specific effect. We found that sdc-2 mutations increased the viability of mnDp66/ mnDp66 males to 52%. Because this suppression is comparable to that achieved by sex-1, sex-1 mutations likely suppress most of the male-specific lethality caused by mnDp66, which is consistent with region 1 not being able to repress xol-1 well in the absence of sex-1.

Results from a different set of experiments are also consistent with region 1 being dependent on sex-1. If the two elements act together to repress xol-1, one expectation might be that loss of one would completely disrupt xol-1 transcriptional repression, such that a further elimination of the other element would not enhance the effect. However, the effects caused by a reduction, rather than complete elimination, of the activity of one element should be enhanced by a reduction in the activity of the other. We found that sex-1 and region 1 behaved accordingly. Although region 1 deletions failed to enhance the phenotype caused by homozygous sex-1 mutations (as discussed above), they did enhance the phenotypes caused by a partial loss of sex-1 function. For instance, hermaphrodites missing one copy of each region (mnDf5/ + + sex-1; Table 1) were slightly Dpy, even though a one-copy deletion of each region by itself caused no phenotype. Also, a deletion of region 1 enhanced the effects caused by a reduction in sex-1 copy number. While yDF20/ + + sex-1 hermaphrodites missing one copy of sex-1, region 2, and region 3, are slightly viable (29%), yDF17/ + + sex-1 hermaphrodites missing one copy of sex-1 and regions 1, 2, and 3, are completely...
<table>
<thead>
<tr>
<th>Regions duplicated</th>
<th>Suppressor mutation</th>
<th>Relevant genotype</th>
<th>Observed/expected Xo males viability (%)</th>
<th>Observed/expected hermaphrodites viability (%)</th>
<th>Total number of embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>—</td>
<td>him-8</td>
<td>286/286 (100)</td>
<td>563/563 (100)</td>
<td>976</td>
</tr>
<tr>
<td>—</td>
<td>sex-1</td>
<td>sex-1</td>
<td>NA</td>
<td>748/1150 (65)</td>
<td>NA</td>
</tr>
<tr>
<td>—</td>
<td>sdc-2</td>
<td>sdc-2</td>
<td>289/982 (29)</td>
<td>1446/1906 (76)</td>
<td>3319</td>
</tr>
<tr>
<td>Dp(1), Dp(1)</td>
<td>—</td>
<td>rmDp66/rmDp66; him-8; sex-1 (+)</td>
<td>287/534 (56)</td>
<td>313/1037 (30)</td>
<td>1806</td>
</tr>
<tr>
<td>Dp(1), Dp(1)</td>
<td>sex-1</td>
<td>rmDp66/rmDp66; him-8; sex-1</td>
<td>172/323 (52)</td>
<td>266/648 (41)</td>
<td>1123</td>
</tr>
</tbody>
</table>

NA, not applicable.

* Numbers in parentheses refer to the regions diagrammed in Figure 2.

** rmDp66 is attached to chromosome II and is homozygous in the strains indicated.

† All numbers in experiments involving rmDp66 were normalized to him-8. The actual viability of him-8 is 87% (849 live adults per 976 total embryos) and 34% of the progeny are male (286 males per 849 total adults). To calculate the number of expected males, the total number of embryos laid by mothers of the indicated genotype was multiplied by (0.87)(0.34). Similarly, the number of expected hermaphrodites was calculated as the total number of embryos multiplied by (0.87)(0.66).

The ratio represents the number of adult observed to number of embryos counted.

* † Actual genotype was dpy-6 sdc-2(y93). sdc-2(y93) causes no significant lethality of its own (Carmi et al. 1998).
Although sex-1(y263)/+ hermaphrodites are wild type, sex-1(y263)/sdc-2(y74) hermaphrodites are Egl and although yd20/+ hermaphrodites are wild type, yd20/+ + sdc-2(y74) hermaphrodites are very Dpy and Tra with significantly reduced viability (57%; Table 6). Even the weak sdc-2 mutation, y93 (Nusbaum and Meyer 1989), which causes no lethality on its own, enhances yd20; yd20/+ + sdc-2(y93) hermaphrodites are slightly Dpy and have slightly reduced viability (87%).

Finally, a weak sex-determination mutation, tra-2(n1106), also enhances sex-1. While the majority of n1106 hermaphrodites are fertile, the majority of tra-2(n1106); sex-1(y263) hermaphrodites are sterile, suggesting enhanced masculinization. With sex-1(y263), the animals are otherwise sterile that a homozygous strain cannot be maintained. Thus mutations in dosage-compensation and sex-determination genes strongly enhance signal element mutations.

The enhancement of signal element mutations by downstream mutations in dosage-compensation and sex-determination genes is consistent with partial derepression of xol-1 in cells with an intermediate X signal. Alternatively, the data may be interpreted as evidence for cell interactions that occur after the activity state of xol-1 has been set. Such interactions might reduce differences between cells, making it look as if xol-1 were at intermediate levels, even if the initial level of xol-1 in each cell were either high or low. This possibility is likely to contribute to the interaction between tra-2 and sex-1 because nonautonomy in sex determination had been previously demonstrated (Villeneuve and Meyer 1990; Hunter and Wood 1992; Kuwabara et al. 1992; Perry et al. 1993). Because current data suggest that dosage compensation is a cell-autonomous process, the dosage compensation phenotypes are likely to reflect xol-1 levels more directly than the sexual phenotype of the animal.

To analyze more precisely the state of dosage compensation in animals with an intermediate X signal, we examined individual cells. We explored the localization of two components of the dosage compensation complex, SDC-2 and DPY-26, in sex-1 mutant hermaphrodites. In wild-type animals, SDC-2 and DPY-26 localize to X chromosomes specifically in XX animals, where low xol-1 levels lead to activation of dosage compensation (Lieb et al. 1996; Dawes et al. 1999). This localization is manifested as a punctate nuclear staining pattern in wild-type XX but not XO embryos (Figures 4, A and B and 5, A and B). Previous studies already suggested that xol-1 activity may be set at an intermediate level in sex-1 mutants because not all XX animals die in this background. However, the incomplete lethality could reflect either intermediate levels of xol-1 in individual cells or mosaic activation of xol-1 in some but not other cells. The levels of SDC-2 protein are likely to reflect the levels of xol-1 activity in these mutants, because sdc-2 acts directly downstream of xol-1 in the sex-determination and dosage-compensation pathway. The level of

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**TABLE 6**

**Interactions between signal elements and downstream sex-determination and dosage-compensation genes**

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>Phenotype</th>
<th>XX Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sex-1(y263)</td>
<td>Dpy, Egl, Tra</td>
<td>65</td>
</tr>
<tr>
<td>sex-1(gm41)</td>
<td>Dpy, Egl, Tra</td>
<td>37</td>
</tr>
<tr>
<td>sdc-1(n485)</td>
<td>Dpy, Egl</td>
<td>&gt;90</td>
</tr>
<tr>
<td>sex-1 sdc-1(n485)</td>
<td>Dead or sterile</td>
<td>NA</td>
</tr>
<tr>
<td>sdc-2(y74)/+</td>
<td>Wild type</td>
<td>100</td>
</tr>
<tr>
<td>sdc-2(y74)/sex-1(gm41)</td>
<td>Egl</td>
<td>NA</td>
</tr>
<tr>
<td>sdc-2(y93)/+</td>
<td>Wild type</td>
<td>100</td>
</tr>
<tr>
<td>yd20/+</td>
<td>Wild type</td>
<td>99</td>
</tr>
<tr>
<td>sdc-2(y93)/yd20/+</td>
<td>Wild type to slightly Dpy</td>
<td>87</td>
</tr>
<tr>
<td>sdc-2(y74)/yd20/+</td>
<td>Dpy, Egl</td>
<td>57</td>
</tr>
<tr>
<td>sdc-3(y129)/+</td>
<td>Dpy, Egl</td>
<td>28</td>
</tr>
<tr>
<td>sdc-3(y129); sex-1+</td>
<td>Dead</td>
<td>0</td>
</tr>
<tr>
<td>tra-2(n1106)</td>
<td>Egl, few Tra</td>
<td>NA</td>
</tr>
<tr>
<td>tra-2(n1106); sex-1</td>
<td>Tra, sterile</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, not applicable.

* Data from Carmi et al. (1998).

* Data from Villeneuve (1989).

* Dpy non-Unc recombinants were picked from sex-1(y263) unc-9+/++; sdc-1 or sex-1(gm41) unc-3+/++; sdc-1. Because sdc-1 is a maternal effect gene, all Dpy non-Unc progeny should be sex-1+/+; sdc-1 sex-1 m-++, where m is unc-9 or unc-3. Sixteen Dpy non-Unc hermaphrodite progeny were cloned, but no homozygotes were found. However, about one-fourth of the Dpy non-Unc hermaphrodites produced no live progeny (4 of 16 for gm41, 3 of 16 for y263), suggesting that the double mutants were either sterile or dead.

* The non-Rol non-Unc hermaphrodite progeny from a cross between sex-1(gm41) males and unc-2 sdc-2(y74); yEx68 [rol-6(d); sdc-2(+)] were Egl, while hermaphrodite cross-progeny from an analogous cross to wild-type males were wild type.

* See Table 1.

* A cross between yDp14/++; unc-2 sdc-2(y93 and y74) XO males and yDp20/unc-1 dpy-3 XX hermaphrodites yielded 220 and 235 Unc-2 (yDp20/unc-2 sdc-2) and 762 and 1227 non-Unc non-Dpy-3 (yDp14/++; yDp20/unc-2 sdc-2, yDp14/++; unc-1 dpy-3/unc-2 sdc-2, and unc-1 dpy-3/unc-2 sdc-2) hermaphrodite cross-progeny, respectively. The viability of the yDp20/unc-2 sdc-2 hermaphrodites was calculated as [3 (number of Unc-2 hermaphrodites) / (number of non-Unc non-Dpy-3 hermaphrodites)] × 100%.

* Data from DeLong et al. (1993).

* No live Unc adult progeny were recovered from sdc-3(y129) unc-76(e911)/++; sex-1(y263 or gm41) mothers.

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between y129 and sex-1. sdc-1 is another maternal effect gene (Villeneuve and Meyer 1987, 1990) that appeared to interact with sex-1. sex-1 sdc-1 homozygous progeny from heterozygous mothers failed to produce live progeny (see Table 6 legend), suggesting that sdc-1 and sex-1 mutations also caused synergistic lethality.

The enhancement of signal element mutant phenotype by sdc-2 mutations was even more striking. sdc-2(y74) is a strong mutation that causes 97% lethality in hermaphrodites but is fully recessive, such that y74/+ hermaphrodites are wild type (Nusbaum and Meyer 1989).
C. elegans Sex Determination Signal

**Figure 4.** Localization pattern of the dosage compensation protein SDC-2 in wild-type and sex-1 mutant embryos. All panels are false-color confocal images of embryos stained with antibodies against SDC-2, shown in green, and propidium iodide (PI), shown in red. (A and B) SDC-2 localizes to the X chromosomes of wild-type XX embryos, exhibiting the characteristic punctate staining pattern. (C–F) SDC-2 also exhibits a punctate pattern in sex-1 mutant embryos, but the staining is fainter and less distinct, implying less SDC-2 on X. Because of the reduced SDC-2 protein level in sex-1 mutants, SDC-2 signal was enhanced during imaging to demonstrate the punctate pattern more readily.

SDC-2 was uniformly reduced in all sex-1 mutant cells, and the detectable SDC-2 appeared consistently localized to X (Figure 4, C–F). These results suggest that most cells had an intermediate level of xol-1 activity and exhibited partial activation of dosage compensation.

In contrast to SDC-2, DPY-26 appeared variably X-localized in sex-1 mutant embryos. In some nuclei DPY-26 was clearly punctate and resembled the wild-type X chromosome staining pattern and in some nuclei the protein was partially diffuse, while in others it was completely diffuse (Figure 5, C–F), suggesting that a mosaic pattern for the X localization of DPY-26 results from reduced SDC-2 levels.

**DISCUSSION**

**Assessing X-chromosome dose in C. elegans:** In this article we investigated how the primary sex determination signal of C. elegans amplifies a twofold difference in X-chromosome dose between the sexes, producing a greater than twofold difference in the activity level of the switch gene xol-1 (Rhind et al. 1995), which controls both sex determination and dosage compensation. Our results show that C. elegans amplifies the sex-determination signal using at least two different strategies: multiple X-linked repressors that act synergistically to reduce xol-1 levels in XX animals, and two levels of xol-1 regulation, transcriptional and post-transcriptional. Together, these mechanisms ensure the correct and stable choice of sexual fate.

Our results demonstrate that the use of a multigenic system to communicate the dose of X chromosomes to xol-1 produces strong repression of xol-1 through the combined action of individual components of the system. We found that twofold changes in the dose of multiple elements, from one to two copies in males and from two to one copy in hermaphrodites, were generally necessary to produce sexual fate transformations. The severity of phenotypes increased as the dose of more elements was changed, indicating that individual X elements exert only partial repression on xol-1, but their effects combine to produce full xol-1 repression. In males, the copy number of at least two elements had to be increased to the hermaphrodite dose to cause masculinization and lethality. A reduction in the copy number of four elements in XX animals was required for complete transformation into the male fate.

Our results offer a possible explanation for the persistence of multiple signal elements in C. elegans. Despite the fact that a one-copy change of any one element has no effect on sexual fate, our results clearly demonstrate that all signal elements contribute significant repression to the X signal. Thus, each element is important to the signal as a whole, even if individual point mutations appear to have no deleterious effects. Full xol-1 repression requires the action of all elements.
Our results also demonstrate that the two levels of xol-1 regulation are essential and act synergistically to repress xol-1. Disruption of either the transcriptional or post-transcriptional mechanism alone can lead to dosage compensation defects, including lethality, in hermaphrodites, indicating that the pathways are not redundant. Furthermore, in hermaphrodites and males, synergistic effects are observed when the doses of elements involved in both forms of xol-1 regulation are simultaneously changed. Thus the two regulatory mechanisms increase the fidelity of the signal by further increasing the difference in xol-1 levels between the sexes.

At this time we do not have sufficient information to determine whether one mechanism of repression is more effective than the other, but our data suggest that both mechanisms can exert strong repression on xol-1. So far mutations in the transcriptional repressor sex-1 result in the strongest dosage-compensation and sex-determination defects observed for any one homologous X signal element mutation. By comparison, homozygous mutations in the post-transcriptional repressor fox-1 cause no deleterious effects on hermaphrodites. These results demonstrate that certain individual signal elements are more effective at repressing xol-1 than others but do not necessarily reflect the relative importance of the mechanism of repression that each gene uses. When provided at high-enough copy number, elements involved in either repression mechanism can strongly repress xol-1 and kill males. However, we still do not know the relative effectiveness of each mechanism at the signal element levels found in wild-type males and hermaphrodites.

Our results also demonstrate that both transcriptional and post-transcriptional signal elements repress xol-1 to some extent in males, even though xol-1 must remain active in XO animals. We showed that fox-1 mutations suppress the male lethality caused by the increased dose of sex-1, and sex-1 mutations, similarly, suppress the male lethality caused by the increased dose of regions 2 and 3. Thus both fox-1 and sex-1 repress xol-1 in males, and the alleviation of this repression can counteract increased repression brought on by the increased dose of other elements.

In summary, it appears that both xol-1 repression mechanisms are necessary and potent, acting synergistically to communicate and amplify the X signal and allowing a twofold difference in the dose of regulators to stably determine sexual fate.

Transcriptional regulation of xol-1 by sex-1 and region 1: Our experiments are most consistent with region 1 being partially dependent on sex-1 for repression of xol-1. These results fit nicely with the molecular biology of SEX-1 as a nuclear hormone receptor (NHR) homolog (Carmi et al. 1998). NRHs are transcriptional regulators that can either repress or activate transcription depending on the presence of additional factors (Horwitz et al. 1996). One possibility is therefore that region 1 encodes a cofactor for SEX-1.

SEX-1 is most closely homologous to retinoic acid receptors (RARs), which can act as repressors in association with several factors (Minucci and Ozato 1996). RARs often associate with retinoid X receptors, activating transcription when bound to ligand but repressing transcription when unliganded. In some cases, RARs can repress transcription by interfering with components of the basal transcriptional machinery (Horwitz et al. 1996). In other cases, RARs repress transcription through association with corepressors like NCoR (Hoerlein et al. 1995) and SMRT (Chen and Evans 1995). Ligands most often activate transcription, but there is at least one example in the literature of a ligand-bound RAR acting as a repressor (Desbois et al. 1991). Other classes of transcription factors may also interact with RARs. For example, the Drosophila activator NHR αFtz-F1 has been shown to associate with Fushi tarazu, a homeodomain transcription factor (Guichet et al. 1997; Yu et al. 1997).

Given all the interactions that have been observed with other NRHs, it seems plausible that SEX-1, too, would require corepressors and that region 1 could encode one of them. In that case, region 1 would enhance repression by SEX-1 but could also repress transcription in the absence of sex-1 through association with additional factors acting at the xol-1 promoter, consistent with its having some SEX-1-independent effects. Alternatively, if SEX-1 requires a ligand for repression, region 1 could be involved with ligand production, accounting for its dependence on sex-1; the independent effects of region 1 could be mediated by another NRH acting at the xol-1 promoter. All these possibilities are consistent with our data.

Different responses to intermediate signal between males and hermaphrodites: Although the qualitative responses to changes in the dose of signal elements were similar between males and hermaphrodites, we found some quantitative differences between the sexes. One difference is that while a twofold increase in the copy number of two signal elements is generally sufficient to cause dosage-compensation defects including lethality in males, a twofold decrease in the copy number of two signal elements in hermaphrodites is generally harmless. There may be several reasons for this difference. First, males may be more sensitive than hermaphrodites to changes in the dose of X signal elements because the proportional change in signal element copy number is greater relative to the total number of signal elements present in a male. Second, the single copy of xol-1 found in males may be more sensitive to changes in the dose of signal elements than the two copies present in hermaphrodites. A third possibility is that males are more sensitive to dosage-compensation defects than hermaphrodites, perhaps because underexpression of X-linked genes is more lethal than overexpression. If that were the case,
small increases in the signal could kill males, but similar decreases in the signal would not kill hermaphrodites.

A second difference between the sexes is the lack of reciprocity in the phenotypic consequences of signal element dose changes. While hermaphrodites exhibit masculinization in response to decreases in the signal, males do not exhibit feminization in response to increases in the signal. If males are indeed more sensitive to dosage-compensation defects than hermaphrodites, the lack of feminization may reflect the fact that all feminized males are dead due to underexpression of X-linked genes. Alternatively, the difference may arise from feedback between dosage compensation and sex determination (DeLong et al. 1993). It has been previously demonstrated that mutations in dosage-compensation genes can have a feminizing effect on intersexual animals. Conversely, the partial activation of dosage compensation in males with an increased X signal may have a masculinizing effect on the animals. Recent work suggests a possible molecular mechanism for feedback involving sdc-2 (H. Dawes and B. J. Meyer, unpublished results). In wild-type XX animals, SDC-2 represses her-1 by binding to its promoter (Dawes et al. 1999), thereby promoting hermaphrodite sexual differentiation. SDC-2 also localizes to the two X chromosomes and recruits other dosage compensation proteins to X to activate dosage compensation (Dawes et al. 1999). In contrast, in wild-type males, sdc-2 is repressed by xol-1 so that it cannot repress her-1 or initiate dosage compensation. If in XO animals with an increased X:A ratio xol-1 is partially repressed, the lower levels of xol-1 may lead to increased levels of sdc-2. However, this extra SDC-2 protein would be recruited to X and stabilized by the ubiquitous dosage-compensation proteins (Chuang et al. 1994, 1996; Lieb et al. 1998), so that it would not be available to repress her-1. As a result, the male fate would be promoted.

Comparison of two multigenic signaling systems, C. elegans and Drosophila: As in C. elegans, the primary signal for both sex determination and dosage compensation in Drosophila is the X:A ratio (Bridges 1921). Drosophila also uses a multigenic system to communicate X dose to its target gene, Sxl, but employs a different strategy to stabilize the choice of sexual fate (Cline and Meyer 1996). The cellular and organismal consequences arising from this difference are discussed below.

While signal elements in C. elegans regulate xol-1 both transcriptionally and post-transcriptionally, only one mechanism of regulation, transcriptional, has thus far been found to affect the initial regulation of Sxl (Keyes et al. 1992). As discussed above, two mechanisms of regulation in C. elegans stabilize the choice of sexual fate. In Drosophila, this stabilization is achieved by an autoregulatory loop that acts after the signal at the level of splicing (Bell et al. 1991). This autoregulation is only activated in XX females, locking Sxl into an active state. In males, the 1X dose of signal elements fails to activate Sxl, so the autoregulatory loop is never employed and Sxl is locked into an inactive state. Sxl therefore acts as a true switch for sexual fate: even when the signal is at an intermediate level, the final state of Sxl is either fully on or fully off at the level of individual cells.

The different strategies used in each species to stabilize the signal determine the possible cellular consequences of an intermediate signal. In Drosophila, because Sxl is always fully on or fully off, individual cells adopt either the female or the male modes of sex determination and dosage compensation but can never become intersexual as a consequence of signal ambiguities. As a result, mutations in downstream sex-determination and dosage-compensation genes cannot enhance the phenotypes caused by mutations in signal elements. Furthermore, because sex determination in Drosophila is cell autonomous (Morgan 1914), an intermediate signal can result in the development of mosaic intersexes: animals consisting of a mixture of male and female cells. Our experiments reveal a different scenario in C. elegans, where an intermediate signal results in cells with intermediate sexual phenotypes. This intersexual cellular fate is manifested by the partial activation of dosage compensation in individual cells, evidenced by the uniform reduction in X localized SDC-2 protein in all cells and the consequent mosaic pattern for the X localization of DPY-26. These intersexual cells may arise because xol-1, unlike Sxl, does not act as a true switch and is expressed at intermediate levels in response to an intermediate signal.

This interpretation would be incorrect only if, counter to our current understanding, dosage compensation were not a cell-autonomous process. In that case, cell interactions occurring after establishment of xol-1 activity states could reduce differences among cells, making it appear as if xol-1 were not a true switch. Whether the intermediate levels of xol-1 or later cell interactions result in the partial activity of dosage compensation, a consequence of the C. elegans system is that, unlike Drosophila, mutations in downstream genes greatly enhance the phenotypes caused by signal element mutations.

A true-switch system may not be desirable in C. elegans because its development follows an invariant cell lineage (Stanton et al. 1983). In Drosophila, although misregulation of Sxl can kill cells due to dosage-compensation defects, the cells can be eliminated and replaced by others, with little or no consequence to the animal (Lish et al. 1990). In C. elegans, however, a loss of one cell could eliminate a whole lineage and whole vital organs.

In summary, C. elegans and Drosophila use similar multigenic regulatory systems to communicate X chromosome dose to their respective target genes, xol-1 and Sxl. However, the different molecular mechanisms used
in each system ultimately lead to dissimilar outcomes at the level of target gene expression and cellular fate.

**Morphogens as dose-sensitive signals:** The action of signal elements parallels the action of morphogens in many ways. While signal elements specify different sexual fates at different concentrations, morphogens specify different cellular and developmental fates at different concentrations, although the actual concentration range for morphogens is unknown. The function of the Drosophila morphogen bicoid has been studied in great detail and illustrates this point well. bicoid forms an anterior-to-posterior gradient in the embryo, activating anterior-specific genes at high concentrations and posterior genes at low ones (Rivera-Pomar and Jekle 1996). The stable specification of anterior or posterior fates, however, requires the combined action of several other morphogens, including caudal and nanos, that also form gradients but in opposite orientation to bicoid. Further stabilization of developmental fate is achieved by auto- and cross-regulation among downstream target genes. Comparison of anterior-posterior fate specification and sex determination reveals common developmental mechanisms for the faithful specification of cell fate, including the use of multiple dose-sensitive regulators and autoregulation by downstream target genes.

The ability of bicoid to activate different genes at different thresholds may be due to the differential affinities of bicoid binding sites in target genes (Driever et al. 1989). Genes harboring high affinity sites for bicoid are activated at low concentrations while genes with low affinity sites are only activated at high concentrations. xol-1 may have similarly evolved signal-element-binding sites that can only be repressed by the XX dose of signal elements found in hermaphrodites.

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