Supporting Information

González et al. 10.1073/pnas.0805993105

DNAS A



Fig. S1. Time course of nascent *SxIPm* transcripts in *D. virilis* determined by in situ hybridizations with a probe specific for *D. virilis SxIPm*-derived premRNA. (*Top*) Surface views of syncytial nuclei in wild-type XX and XY embryos during cycles 13 and 14. (*Bottom*) Views of elongating nuclei of the XY embryos pictured above to monitor progression through the cellularization cycle. Nascent transcripts from sister chromatids can be seen in some nuclei (15, 22).



Fig. S2. Time course of nascent *SxIPm* transcripts in maternal or zygotic sex signal mutants. (*A*) Progeny of homozygous *da*¹ mothers. (*B*) *sisA*¹ mutant embryos from crosses of *y pn cm SxI^{M4} v sisA*¹/*y pn cm SxI^{f1} ct*⁶ *v sisA*¹ females and *y pn cm SxI^{f1} ct*⁶ *v sisA*¹/*Y* males. (*C*) Progeny of mothers carrying *gro*^{E48} germline clones.

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Fig. S3. Time course of nascent transcripts from Sx/Pm-lacZ fusions during cycles 13 and 14 detected by in situ hybridization with a lacZ probe. Surface views of syncytial nuclei. (A) Embryos homozygous for an autosomal $Sx/Pm[\Delta-88Pe]$ -lacZ line. (B) Embryos homozygous for an autosomal $Sx/Pm[\Delta-1.4Pe]$ -lacZ line. Sex was determined by fluorescent detection of endogenous Sx/Pe-derived transcripts. Four independent lines of each transgene were examined, with indistinguishable results. Nascent transcripts from lacZ transgenes were more difficult to detect than those from the endogenous Sx/ locus, resulting in patchier lower-intensity staining.

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Table S1. Percentage of nuclei expressing SxIPm at indicated cell cycle times

0 (5)

89-90 (2)

74 (1)

45-55 (3)

9–11 (3)

46-57 (2)

10 (2)

0 (3)

71 (1)

27 (1)

11-13 (2)

0 (2)

11-16 (2)

0 (3)

Genotype

Dp sc⁺, sisA⁺

XY

ΧХ

XY

ΧХ

XY

ΧХ

XY

wt

sc^{sisB3}

da¹

sisA¹

gro^{E48}

<

	Percentage of nuclei expressing <i>SXIPm</i> , range (number of empryos counted)				
	Cycle 13	Cycle 14, < 5 min	Cycle 14, 5–10 min	Cycle 14, 10–15 min	Cycle 14, 15–20 min
ХХ	12–16 (4)	48–61 (5)	85–91 (5)	100	100
XY	0 (10)	6–9 (5)	33–39 (3)	71–85 (3)	100
XX	0 (2)	12–16 (3)	51–63 (2)	80–90 (2)	100
XY	0 (3)	0 (2)	7–9 (2)	53 (1)	77–79 (2)
XX	0 (8)	7–13 (3)	50–57 (3)	83 (2)	100

8-9 (2)

100

81 (1)

83-88 (2)

21–35 (2)

81-85 (2)

30-32 (2)

54-60 (2)

100

100

100

82–85 (2)

100

79-85 (2)

76-88 (2)

100

100

100

100

100

100

Percentage of nuclei expressing SxIPm, range (number of embryos counted

The percentage of expressing nuclei was determined by counting all nuclei in photographs of embryos. Mean number of cycle 13 nuclei counted/embryo (\pm standard error) = 224 (\pm 26); mean number of cycle 14 nuclei counted/embryo = 432 (\pm 38). For samples listed as 0% expressing, the number in parentheses indicates photographs examined for evidence of expression. At least 10 additional fixed embryos were examined directly for expressing nuclei for all nonexpressing stages. For those listed as 100% expressing, at least one photograph and several other embryos were examined thoroughly for rare nonexpressing nuclei.