x-ray films to cut out the ^{35}S label and record only the ^{32}P signal. The AP-2 oligonucleotide was derived from the human-metallothionein IIa upstream region (*10*).

Cross-linking was performed in crude reticulocyte lysate, which enables proteins that do not bind to DNA to be analyzed. In this method, 2 M KCl (6.6 μl) was added to reticulocyte lysate (10 μl). A portion of this mixture (3.1 μl) was taken out and left untreated at room temperature for 60 min as a control. Glutaraldehyde (1.5 μl of 0.1%) was added to the rest (13.5 μl) of the mixture and it was incubated at room temperature for 60 min. Reactions were stopped by the addition of nine volumes of RIPA buffer (150 mM NaCl, 10 mM tris-Cl, pH 7.4, 1% Triton X-100, 1% sodium deoxycholate, 1 mM EDTA, 1 mM PMSF and 2 μg/ml aprotinin). Proteins were separated by SDS-PAGE and subsequently visualized by fluorography, following treatment of the gel with Amplify (Amersham).

21. The data concerning the dimerization and DNA binding (column a) of the wt, and NH_2 and COOH-terminal deletion mutants was reported in (*12*). Data on the internal deletions was obtained in this report. The DNA-binding data reported in column b for the NH_2- and COOH-terminal deletions is from (*12*) for ΔN165, or otherwise from (*13*).
22. Members of the HLH family of proteins are characterized and defined by a degree of sequence similarity, which enabled a consensus motif to be derived (*8*). The AP-2 HSH does not fit this consensus and is also much larger. However, both the HLH and HSH motifs are defined only by primary sequence and it is therefore possible that these proteins may share similarities on a tertiary structural level.
23. Three c-Jun–AP-2 fusions were constructed by cleaving pHJ19-MUT14, pHJ19-MUT16, and pHJ19-MUT17 (*15*) with Cla I and Kpn I and inserting an ~750-bp fragment of AP-2 that spanned from the Pst I site at nt 892 of AP2-9 to the Kpn I site in the 3′ polylinker sequence (*10*) with a Cla I–Pst I adaptor oligonucleotide. The sequence of the adaptor, which also contains a Sca I site, is ATCGATGCAGTACTGCCTGCAG. These fusions were then transferred into Pst I–Eco RI digested full-length c-Jun expression vector pHJ40 (*15*) with a restriction fragment that spanned from the Pst I site in c-Jun to the Eco RI site at the 3′ end of the AP-2 sequences, to generate JunL0HSH, JunL1HSH, and JunL2HSH, respectively. JunL2HSX is identical to JunL2HSH except that it lacks 12 bp between nt 1231 and 1242 of AP2-9. All constructs were verified by sequencing and Western blotting. The constructs were transcribed in vitro with T3 RNA polymerase (Stratagene) and then translated and assayed as in (*20*). The AP-1 oligonucleotide was derived from the human metallothionein IIa upstream region (*5*). Autoradiography of SDS-PAGE gels indicated that equivalent amounts of each protein were synthesized (*13*).
24. The JunL2HSH, HJ40, (*6*) and pBluescript SK$^+$ (Stratagene) plasmids were transformed into the *Escherichia coli* TG1 strain, and protein was prepared by the inclusion body method (*15*). AP-2 is not efficiently made with this procedure and was therefore obtained with the urea method (*10*). DNase I footprinting reactions (*25*) were performed with a ^{32}P 5′ end-labeled Ava I–Pst I fragment from LS-ES-TK (*26*).
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27. We are indebted to R. Turner, V. Baichwal, G. Gill, S. Jackson, and N. Mermod for discussion, encouragement, and criticism throughout the course of this work; and to D. Bohmann both for the c-Jun plasmids and helpful advice at the outset of the project. Supported in part by an NIH grant (R.T.) and a Howard Hughes Medical Institute Postdoctoral Fellowship (T.W.).

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Molecular Nature of the *Drosophila* Sex Determination Signal and Its Link to Neurogenesis

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In 1921 it was discovered that the sexual fate of *Drosophila* is determined by the ratio of X chromosomes to sets of autosomes. Only recently has it been found that the X chromosome to autosome (X:A) ratio is communicated in part by the dose of *sisterless-b (sis-b)*, an X-linked genetic element located within the *achaete-scute* complex of genes involved in neurogenesis. In this report, the molecular nature of the primary sex determination signal and its relation to these proneural genes was determined by analysis of *sis-b*$^+$ germline transformants. The *sis-b*$^+$ function is confered by protein T4, a member of the helix-loop-helix family of transcription factors. Although T4 is shared by *sis-b* and *scute-α*, the regulatory regions of *sis-b*, which control T4 expression in sex determination, are both separable from and simpler than those of *scute-α*, which control T4 expression in neurogenesis. Dose-sensitive cooperative interactions in the assembly or binding of *sis*-dependent transcription factors may directly determine the activity of the female-specific promoter of *Sex-lethal*, the master regulator of sexual development. In this model there is no need to invoke the existence of analogous autosomal negative regulators of *Sex-lethal*.

THE SOMATIC SEXUAL FATE OF THE fruit fly is determined by the dose of at least two X-linked *sisterless* loci (*sis-a* and *sis-b*) and by the interactions of these genes with their regulatory target *Sex-lethal (Sxl)* (*1*). *Sxl* is a female-specific gene that coordinates all aspects of sexual differentiation including the vital process of X chromosome dosage compensation. Increases in *sis* gene dose increase the probability that a cell will express the female-specific sex determination and dosage compensation activities of *Sxl*, while decreases have the converse effect. What feature of the *sis* genes allows them to serve as indicators of the X chromosome to autosome (X:A) ratio? How does *Sxl* reliably distinguish the twofold difference in *sis* gene dose that defines the chromosomal sex of the fly? Is the role of the *sis* genes in development limited to sex determination? The answers to these questions are fundamental to understanding how sexual dimorphism is controlled in *Drosophila* and how that control has evolved.

The location of *sis-b* within the extensively studied *achaete-scute* complex (AS-C) (*1, 2*) has facilitated analysis of the sex determination signal. At the same time, it has raised the question of how a sex determination signal element might be functionally related to a gene complex that is directly involved in the development of the peripheral nervous system (*3*). The AS-C is composed of a few small transcription units flanked by an enormous amount of regulatory DNA (*4–7*) (Fig. 1). The initial characterization of *sis-b* indicated that most, but not all, *sis-b* activity mapped to a 22-kilobase (kb) portion of the complex that contains the transcription unit (T4) of the *scute-α(sc-α)* gene (*1*). Subsequent genetic analyses of point mutations in the protein coding region of T4 suggested that the protein product of this gene participates in the sex determination functions of *sis-b* (*2*), although the picture was clouded by the variable, and sometimes surprisingly weak, *sis* phenotypes of lesions that eliminated T4 protein activity. Here we use *P* element mediated transformation with genomic *sis-b* DNA to address the question of how the sex determination signal in *Drosophila* is related to neurogenesis and whether a *sis-b* encoded protein is part of that signal.

Three constructs that contained the intact T4 transcription unit with varying amounts of flanking genomic DNA (Fig. 1) were found to have *sis-b*$^+$ activity by the criteria listed in Fig. 2 (*8*). The T4$^+$ transgenes complemented the female-specific, temperature-sensitive lethal allele *sis-b*$^{sc3\text{-}1}$ (Table 1A). Of the 14 lines tested, only *P*[B5]21 failed to fully complement the *sis-b* mutation, and even it rescued most of the mutant females. The ability of extra copies of one *sis* locus to partially compensate in females for decreases in the activity of another *sis* locus is a distinguishing feature of these genes. The *P*[B5]T4$^+$ transgenes displayed this additive behavior, rescuing females that carried a partial loss-of-function *sis-a* point mutation over a deletion of the *sis-a* locus (Table 1B). This test demands much higher *sis-b*$^+$ activity than the *sis-b*$^{sc3\text{-}1}$ complemen-

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tation assay (Table 1A). Rescue observed with one (6 to 15%) or two (50 to 80%) transgenes is comparable to that reported for one (5 to 7%) or two (70%) copies of much larger chromosomal duplications that carried $T4^+$ (*1*). The $T4^+$ transgenes mimic sis^+ chromosomal duplications in that they can cause male lethality by activating *Sxl* in haplo-X flies. The *P*[B5]$T4^+$ transgenes substantially reduced male viability in the presence of an extra copy of $sis\text{-}a^+$ inserted into the Y chromosome (Table 2). This male-lethal effect was completely suppressed by *Sxl* mutations (*9*).

To test whether the protein product of the T4 transcription unit is responsible for the $sis\text{-}b^+$ activity of the transgenes, two modified transgenes were constructed: one carried a small (4-base-pair) frameshift in T4; the other a larger deletion-insertion in the T4 coding sequence (Fig. 1). Both changes in the transforming DNA abolished $sis\text{-}b^+$ activity as judged by the $sis\text{-}b^{sc3\text{-}1}$ complementation test (Table 1A) and the male-lethal test (Table 2), indicating that in the genomic regions carried by the transgenes, the T4 product alone is responsible for $sis\text{-}b^+$ activity.

Although the transgenes had high amounts of $sis\text{-}b^+$ activity, they were devoid of *sc*-α function. The transgenes complemented neither the extreme bristle defects of $In(1)ac^3\ sc^{10\text{-}1}$ mutants, nor the much weaker bristle defects caused by $sis\text{-}b^{sc3\text{-}1}$ (*10*). This failure of the transgenes to provide *sc*-α function is not surprising because many strong *scute* mutations map far from the region included in these transgenes (Fig. 1). Indeed, aberrations as far as 50 kb downstream and 22 kb upstream of T4 can alter the sensory bristle pattern of adults (*4, 5*). Further evidence for the existence of distinct regulatory regions for *sc*-α and *sis-b* comes from our analysis of $sis\text{-}b^{sc3\text{-}1}$, an allele that affects sex determination much more strongly—and with a different temperature dependence—that it affects neurogenesis. The temperature sensitive period for the female-lethal effect (*11*) corresponds remarkably well with the brief early embryonic pulse of T4 mRNA that occurs between nuclear cycles 9 and 14 (*12*).

For *sis-b* and *sc*-α, studies of sex determination and neurogenesis have converged to provide a fascinating example of how a single protein is used in two seemingly unrelated processes as a consequence of the evolution of cis-acting regulatory information. One set of information governs the spatially restricted expression of T4 in neurogenesis, and a second, much smaller set (conceivably a subset of the first), governs the temporally restricted but spatially uniform expression of T4 in sex determination.

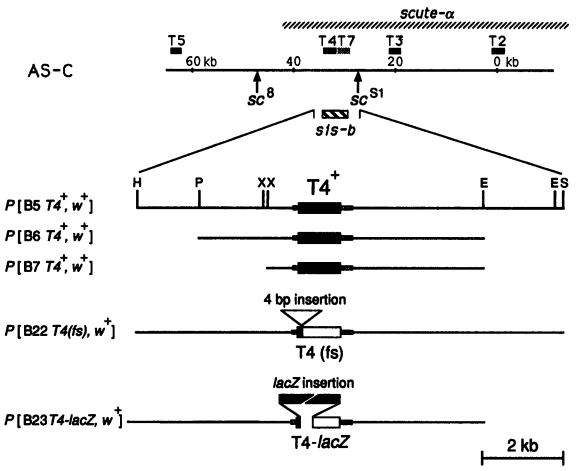

Fig. 1. AS-C genomic DNA tested for *sis-b* function. A diagram of most of the *achaete-scute* complex (AS-C) is at the top of the figure. Four transcription units of the complex, T2 to T5 are shown above the molecular coordinates of the AS-C (*4, 6*). Also shown is an uncharacterized transcription unit T7 that has been reported, but seems not to be important for the neurogenic functions of the AS-C (*7*). The *scute*-α locus, defined as the region within which mutational breakpoints confer moderate to strong *scute* bristle phenotypes, is shown as a hatched bar above the complex (*4, 5*). The 22 kb between the sc^8 and sc^{S1} breakpoints (arrows) is the region that was originally identified as containing most of the $sis\text{-}b^+$ activity (*1*). The DNA fragments from this region tested for *sis-b* function are shown at an expanded scale below the diagram of the AS-C (*24*). The extent of the shortest rescuing fragment B7 is indicated to scale as a hatched bar labeled *sis-b*, just below the AS-C diagram. The thick portions of the horizontal lines mark the T4 transcription unit with the coding sequences shown as large filled rectangles. All genomic fragments originated from λ*sc64* (*4*) and were ligated into the w^+ Casper *P* element vector (*25*). Germline transformants were obtained by standard methods (*26*). The $T4^+$ constructs *P*[B5], *P*[B6], and *P*[B7] contain, respectively, fragments of 10.5 kb [Hind III–Sal I (37.1 to 26.6)], 6.7 kb [Pst I–Eco RI (35.5 to 28.8)], and 5 kb [Xba I–Eco RI (33.8 to 28.8)]. The $T4^-$ construct *P*[B22] was derived from *P*[B5] by filling in a Bgl II site, which generated a frameshift after amino acid 34 (T4 encodes 345 amino acids). The T4-*lacZ* insertion in *P*[B23] was created by replacing the Bgl II fragment that encodes T4 amino acids 35 to 185 with an in-frame *lacZ* gene from pMC1871 (*27*). The left end of the insert in *P*[B23] is a vector-derived Eco RI site near coordinate 37.4.

♀ ***sisterless*** **gene dose**

		effect	suppressed by
two copies $sis\text{-}b^+$	two copies $sis\text{-}a^+$	wild-type	—
one copy $sis\text{-}b^+$	one copy $sis\text{-}a^+$	lethal	extra copy $sis\text{-}b^+$ or $sis\text{-}a^+$
no copies $sis\text{-}b^+$	two copies $sis\text{-}a^+$	lethal	extra copy $sis\text{-}b^+$
two copies $sis\text{-}b^+$	no copies $sis\text{-}a^+$	lethal	extra copy $sis\text{-}b^+$

♂ ***sisterless*** **gene dose**

		effect	suppressed by
one copy $sis\text{-}b^+$	one copy $sis\text{-}a^+$	wild-type	—
one copy $sis\text{-}b^+$	two copies $sis\text{-}a^+$	viable	—
two copies $sis\text{-}b^+$	one copy $sis\text{-}a^+$	viable	—
two copies $sis\text{-}b^+$	two copies $sis\text{-}a^+$	lethal	Sxl^-

Fig. 2. Relevant genetic interactions of the *sisterless* genes (*1*). The female lethal effect of decreases in *sis* gene dose are due to the failure to activate *Sxl* and the consequent establishment of the male mode of X chromosome dosage compensation. Conversely, the male lethality observed with increased *sis* dose is due to the activation of *Sxl* and the subsequent repression of the male dosage compensation functions.

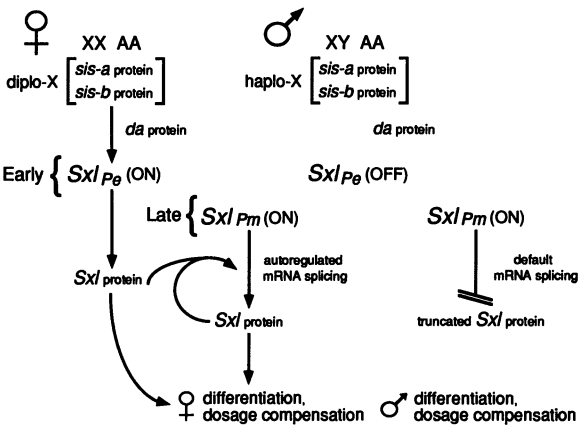

Fig. 3. Model of the initiating events in somatic sex determination. The female-specific protein products of *Sxl* are key determinants in the regulatory gene heirarchy that controls sexual fate (*1, 28*). When present they impose female development; when absent, male development follows. In XX AA embryos, a diplo-X dose of the *sis* products, in combination with maternally supplied *da* protein, transiently activates the *Sxl* early-acting promoter (P_e) (*14, 15*). Transcripts from P_e produce *Sxl* proteins that are required to positively regulate splicing of *Sxl* transcripts that subsequently arise from the *Sxl* constitutive promoter (P_m), which becomes active as P_e shuts off. Productive splicing of the P_m-derived transcripts is then maintained by an autoregulatory loop involving the *Sxl*-encoded proteins (*13*). In male (XY AA) embryos, the haplo-X dose of *sis* products is insufficient to activate P_e; consequently, there is no *Sxl* protein early. For this reason, the transcripts subsequently derived from P_m are spliced to produce a nonfunctional protein product. Some mechanism must operate to keep P_e inactive in both sexes after the onset of dosage compensation (*16*).

How does the primary sex determination signal regulate its target, *Sxl*, at the molecular level, and what does the participation of the T4 protein imply about the mechanism of *sis-b* action? Throughout most of development, the on-off regulation of *Sxl* occurs at the level of RNA splicing, with the active mode being maintained by an autoregulatory feedback loop that involves the *Sxl* protein (*13, 14*); however, the action of the *sis* genes appears to be at the level of *Sxl* transcription instead (Fig. 3). Recent work has indicated that the activation of *Sxl* in response to the X:A ratio occurs early in embryogenesis through effects on a promoter, $P_{establishment}$, that is different from the constitutive promoter, $P_{maintenance}$, which operates throughout most of development (*14, 15*). A female X:A signal activates P_e, while a male X:A signal does not. The subsequent activation of P_m in both sexes is accompanied by a shutdown of P_e as the system becomes unresponsive to the X:A ratio (*16*).

A transcriptional role for the X:A signal is consistent with what is known about its components. Both *sis-b* and *daughterless (da)* encode members of the helix-loop-helix (HLH) class of transcription factors (*6, 17*). *da* is an X:A signal transduction gene (Fig. 3) whose product is required for *Sxl* to respond to the dose of the *sis* genes. (*1, 18*). Some HLH proteins interact to form hetero- and homooligomers (*17*). Thus it is possible that the *da* and *sis-b* proteins interact with each other or with the products of other X:A numerator and signal transducing genes to form an active transcription complex at P_e. We suggest that the remarkable dose sensitivity of the signal elements may be a consequence of both cooperative assembly of the *sis*-dependent transcription factors from their monomeric components and cooperative binding of the assembled factors to P_e (*19*).

What of the autosomal (denominator) component of the X:A ratio? Are there likely to be specific negative regulators of *Sxl* analogous to the *sis* genes of the numerator? Any autosomal factor interfering with the assembly or binding of *sis*-dependent transcription factors at P_e would function as a denominator element in our scheme. There are several precedents for inhibitory interactions among HLH proteins, including *extramacrochaetae* and *hairy*, which negatively regulate the AS-C (*20*); moreover, misexpression of *hairy* can interfere with the X:A mediated activation of *Sxl* (*21*). We expect such negative interactions in the X chromosome counting process to depend on competitive equilibria in protein assembly and binding, rather than on a titration per se that involves sequestration of factors in an all-or-none fashion. On the other hand, there need not be specific autosomal elements analogous to the *sis* genes. The observed affinity of transcriptional activators for nonspecific DNA, coupled with the fact that there is five times as much autosomal as X-linked DNA, might well account for the inhibitory effect of increasing ploidy on the activation of *Sxl*. Similarly, the increased nuclear volume that accompanies increasing ploidy could shift the equilibrium away from the active transcription complex by reducing the concentration of *sis* proteins in the nucleus. For wild-type flies, only the number of X chromosomes differs between the sexes, not the number of autosomes. As a consequence, the genes that function as *sis* elements need only have evolved so that the number of X chromosomes be an unambiguous signal in diploid flies. Apart from a chromosomal pairing model, it is difficult to imagine plausible mechanisms that would not be sensitive to their number relative to the autosomes, rather than to their absolute number, regardless of whether or not those effects can be assigned to specific autosomal genes.

The 1920's view of *Drosophila* sex determination (*22*) as reflecting a "balance be-

Table 1. Rescue of mutant females by $T4^+$ transgenes. (**A**) Complementation of female-specific lethality of *sis-b*$^{sc3\text{-}1}$ by AS-C ($T4^+$) transgenes. Crosses were at 29°C and of the form: *sis-b*$^{sc3\text{-}1}$ *w* / *Binsinscy* ♀♀ X ♂♂ *sis-b*$^{sc3\text{-}1}$ *w* / Y; *P*[(B) w^+] / +. Inserts were present on the first, second, and third chromosomes. Eight *P*[B5], one *P*[B6], six *P*[B7], one *P*[B22], and three *P*[B23] inserts were tested with results similar to those shown. (**B**) Suppression of female-specific lethality of *sis-a* by AS-C ($T4^+$) transgenes. Crosses were as follows at 25°C: (i) construct 2: *y w Df(1)N71, sis-a*$^-$ / *Binsinscy; P*[(B5)T4$^+$, w^+]2 /+ ♀♀ X ♂♂ *y w sis-a* / Y; *P*[(B5)]T4$^+$, w^+]2 / +. The viability reference was *y w sis-a* / *Binsinscy* females; (ii) construct 19: *y w sis-a; P*[(B5)]T4$^+$, w^+]19 /+ ♀♀ X ♂♂ *y w Df(1)N71, sis-a*$^-$ / Y; $X^{d}2^{P}T(1;2)Hw^{bap}$, γ^+Hw^{bap}, *sis-b*$^+$ & *Dp(1,2)v*^{65b}, v^m *sis-a*$^+m^+$ / +. The viability reference was *y w sis-a* males; (iii) constructs 19 and 10: *y w Df(1)N71, sis-a*$^-$ / *Binsinscy; P*[(B5)T4$^+$, w^+]10 ♀♀ X ♂♂ *y w sis-a* / Y; *P*[(B5)T4$^+$, w^+]19 / +. The viability reference was *y w sis-a* / *Binsinscy; P*[(B5)T4$^+$, w^+]10 / + females. *Binsinscy* is the X chromosome balancer: *In(1) sc*^{S1L} *sc*^{8R} + *dl-49* γ^{c4} *w sn*^{X2} *B*. All markers and rearrangements have been described (*1, 23*).

A *P*[(B) w^+] element	Relative viability of *sis-b/sis-b* females *P*[(B)w^+]+	+/+	Reference females *sis-b*/+; *P*[(B)w^+]/+ (*n*)
Wild-type T4			
P[(B5)T4$^+$, w^+]21	70%	2.9%	70
P[(B5)T4$^+$, w^+]19	102%	0.6%	177
P[(B6)T4$^+$, w^+]1	109%	<2.3%	44
P[(B7)T4$^+$, w^+]3	94%	<0.8%	136
Loss-of-function T4			
P[(B22)T4(fs), w^+]1	1.2%	0.2%	421
P[(B23)T4-*lacZ*, w^+]47	<0.8%	<0.8%	130

B	Relative viability of *sis-a/Df(sis-a)* females carrying:	
No transgenes	One copy *P*[(B5)*T4*$^+$, w^+] (*n*)	Two copies *P*[(B5)*T4*$^+$,w^+] (*n*)
<0.1%*	Construct 2: 15% (26) Construct 19: 6% (10) Construct 10: 10% (13)	Constructs 2 + 2: 50% (42) Constructs 19 + 10: 84% (108)

*We have never observed a *sis-a/Df (sis-a)* escaper in my experiment. *n*, number of flies.

Table 2. Male lethality of AS-C ($T4^+$) transgenes in combination with *Dp sis-a*$^+$. Crosses at 22°C: *w/w; P*[T4, w^+]/+ ♀♀ X ♂♂ *v/* v^+ $Y\gamma^+$, v^+ *sis-a*$^+$ γ^+.

P[T4, w^+] line tested	Relative male viability with *Dp(sis-a*$^+$*)**		Females, viability reference (1/2 *n*)
	P[T4, w^+]/+	+/+	
[B5]22	0.3%	87%	379
[B5]20	0.8%	82.5%	400
[B5]19	2.3%	86%	350
[B5]24	4.9%	77%	267
[B5] 3	6.9%	98%	130
[B5]10	14%	90%	315
[B5] 2	25%	96%	236
[B22T4(fs)]	90%	87%	116

*X/O and X/$v^+Y\gamma^+$ males could not be distinguished; however, in control crosses the frequency of X/O males was only 0.44% (5 of 1144).

tween female-determining genes on the X chromosome and male-determining genes on the autosomes" was a formalism to account for the reciprocal influences of X chromosome and autosome dose on sexual development. We now know that the specific X-linked female determiners exist and that at least one, *sis-b*, is a conventional (protein encoding) gene. The male determiners remain a formalism and may never be more than that.

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9. Suppression of the male-lethal interaction by *Sxl* was measured for six $T4^+$ lines in crosses of genotype: *w cm* Sxl^{7B0} *ct* / *w* + + +; $P(T4^+, w^+)$ / + ♀♀ X ♂♂ *l(1)10Ba/v*$^+$$Yy^+$. It is important in such tests to avoid balancer chromosomes while including the appropriate internal controls.
10. Mutant $In(1)ac^3$ sc^{10-1} flies are nearly devoid of bristles because of a rearrangement affecting transcription unit T5 and a point mutation within T4 (*4, 6*). With the exception of an occasional sex-comb tooth, the $T4^+$ transgenes failed to restore any of the macrochaetae that develop normally in $In(1)ac^3$ individuals. In some cases a small fraction of the microchaetae eliminated by the ac^3 (T5) rearrangement were restored.
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Enhancement of HIV-1 Cytocidal Effects in $CD4^+$ Lymphocytes by the AIDS-Associated Mycoplasma

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Coinfection with *Mycoplasma fermentans* (incognitus strain) enhances the ability of human immunodeficiency virus type–1 (HIV-1) to induce cytopathic effects on human T lymphocytes in vitro. Syncytium formation of HIV-infected T cells was essentially eliminated in the presence of *M. fermentans* (incognitus strain), despite prominent cell death. However, replication and production of HIV-1 particles continued during the coinfection. Furthermore, the supernatant from cultures coinfected with HIV-1 and the mycoplasma contained a factor that inhibited the standard reverse transcriptase enzyme assay. The modification of the biological properties of HIV-1 by coinfection with mycoplasma may be involved in the pathogenesis of acquired immunodeficiency syndrome (AIDS).

HIV-1 INFECTION OF EXPERIMENTAL animals, including chimpanzees, has not produced any specific illnesses other than transient lymphadenopathy despite evidence of more than 7 years of persistent viremia. Furthermore, HIV-infected patients often show a wide variation in times of disease incubation and speed of disease progression. It is not known whether any specific infectious agent other than HIV can be responsible for the complex pathogenesis often seen in this disease. One such candidate, initially reported as a virus or virus-like infectious agent, is the mycoplasma *M. fermentans* (incognitus strain) (*1–4*). The organism causes a fatal systemic infection in primates (*5*) and induces necrotizing lesions in the lymph nodes, spleens,

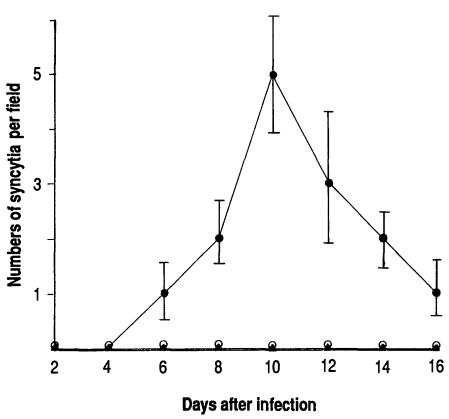

Fig. 1. Inhibition of HIV-1–induced syncytium formation by *M. fermentans* (incognitus strain). We infected A3.01 cells (5×10^7) with (▲) HIV-1 (1×10^5 infectious units) and incognitus strain (1×10^3 infectious units), (●) HIV-1, or (○) incognitus strain. Cells in each culture were incubated at 37°C for 2 hours and then washed once with RPMI 1640 medium. The infectious titer of HIV-1 was previously determined by exposing A3.01 cells to tenfold serial dilutions of HIV-1 culture stock for 2 hours at 37°C. The highest dilution in which the presence of RT activity could be detected after 14 days in culture represented one infectious unit. We grew the incognitus strain in modified SP-4 media and filter-cloned it three times from a single colony on agar plates (*3*). The organisms were washed once and resuspended in RPMI 1640. The titer of incognitus strain after infection of NIH 3T3 cells was determined by an antigen dot blot assay (*3*). The cell cultures were maintained with RPMI 1640 supplemented with 10% fetal bovine serum. Results are the average of the number of syncytia per field (×200) of ten fields examined per culture. The error bars indicate standard deviation of the mean.