

Perspectives

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Reflections on a Path to Sexual Commitment

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IT was with some self-consciousness that I accepted an invitation to reflect on my own work, a long GENETICS article that appeared 20 years ago entitled, "Autoregulatory Functioning of a *Drosophila* Gene Product That Establishes and Maintains the Sexually Determined State" (CLINE 1984). This article was a milestone in a line of work that began with an intriguing maternal effect and led ultimately to an understanding of the fly's sex-determination signal and the self-propagating response of that signal's target. The invitation to reflect on that 1984 article has given me an opportunity to make more explicit the lines of argument that I had not always made clear, writing at a time when authors stressed data presentation and expected readers to infer more of the logic and significance of the arguments. In relating the story of this article, I also emphasize two general points about experimental science. First, the shortest distance between two points is often not the straight line that one might have initially imagined. I certainly did not set out to "solve" fly sex determination. Second, although research stories often appear inevitable in retrospect, the humbling fact is that they almost always involve a great deal of irony and luck. While both of these points have been made by others, I hope an additional illustration might be useful, or at least entertaining.

The pleasure of the wild ride that the fruit fly took me on more than 2 decades ago addicted me to genetics for life. I was doubly fortunate to have had this genetic story unfold just as molecular tools were becoming available to confirm its validity and make the work more accessible to those who might not be genetically inclined. In that connection, I acknowledge a great debt to my former collaborator, the molecular biologist Paul Schedl.

The 1984 article is best known for describing an epi-

genetic (determinative) aspect to the regulation of the X-linked gene that coordinately controls *Drosophila* sexual fate and X chromosome dosage compensation in response to X chromosome dose and for revealing a positive feedback activity of this gene's product that might explain how the active (female) state is maintained (see Figure 1). This gene was appropriately named *Female-lethal* by H. J. Muller in 1960 when he discovered a female-specific lethal allele. I felt compelled to violate protocol in 1978 by changing the gene's name to *Sex-lethal* (*Sxl*) when I isolated a male-specific lethal allele of *Female-lethal* and anticipated the confusion that might otherwise arise.

The 1984 article seems like ancient history to me now, in part because it was necessarily devoid of molecular biology; nevertheless, its conclusions have survived subsequent molecular scrutiny. In the current genomics age with its emphasis on technology and massively parallel approaches, those who have never struggled through a highly focused "pure" genetics article like the 1984 article may not appreciate how much such a low-tech approach can achieve under the right circumstances, even for a subject as complex as metazoan development.

But any lesson that this article might provide regarding the enduring power of classical genetics pales in comparison with that of H. J. Muller's 1932 pure genetics article (MULLER 1932). From mutant phenotypes alone, Muller deduced the various ways in which mutations can affect gene function and discovered X chromosome dosage compensation. One of the greatest ironies in genetics is that this brilliant man discovered *Sxl*, but died 7 years later with no inkling that he had found the master regulator of the dosage-compensation process that he had discovered so many years before. There was simply no place for sex-specific lethality in fly workers' thinking about dosage compensation or sex determination until the *Sxl* story unfolded (see STEWART and MERRIAM 1980; LUCCHESI 1983).

I had the advantage of ignorance coming to the subject of fly sex-specific lethality. I had entered graduate school in 1968 with a biochemistry degree but no back-

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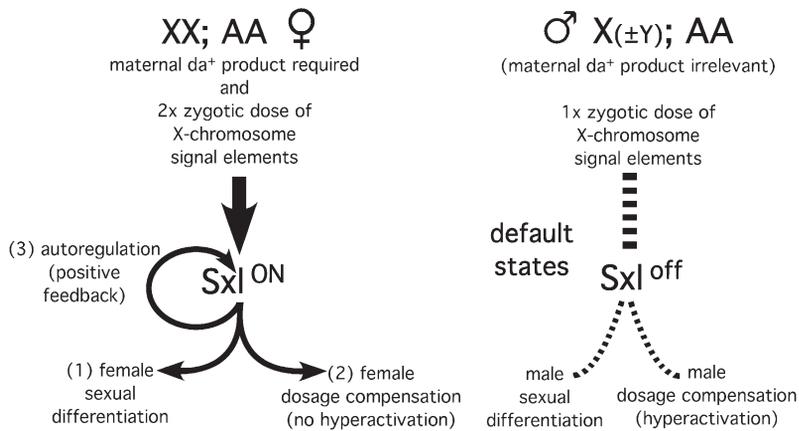


FIGURE 1.—Figure adapted from Figure 5 in CLINE (1984) with the legend taken verbatim: A summary of the functioning of Sxl^+ in *Drosophila* development. Maternal da^+ product and the proper balance ($X/A = 1$) of X-linked and autosomal signal elements in the zygote are required for the functioning of Sxl^+ . Sxl has three general classes of *trans*-acting functions: one that elicits female sex differentiation, probably through interactions with the genes *tra* and *tra-2*; one that suppresses dosage-compensated X-linked gene hyperactivation through interactions with the known autosomal male-specific lethal genes and other genes whose identity is not yet known; and one, demonstrated for the first time here, that can activate Sxl^+ itself, even in the absence of maternal da^+ activity. In the absence of Sxl^+ activity, male development and an increased rate of dosage-compensated gene expression ensue.

ground in genetics, since in those days biochemistry mentors generally considered genetics irrelevant to their discipline. For my Ph.D., I studied how and when marine bacteria generate light, initially unconcerned that the system I had chosen lacked the prerequisites for genetic analysis. Nevertheless, I began to rely on mutations, especially temperature sensitives, and did genetics the hard way, for example, using *in vitro* complementation tests of enzyme extracts to determine allelism. I switched to *Drosophila* as a postdoc in part because I sought a *bona fide* genetic system and was stimulated by lectures on fly development by D. Suzuki, J. Postlethwaite, R. King, and W. Gehring. I joined the University of California at Irvine's Center for Pathobiology (*not* a germ warfare lab!) in 1973, having learned only shortly before how to distinguish male and female flies in a wonderful Cold Spring Harbor neurophysiology course taught by W. Pak and S. Benzer's talented postdocs.

The fondness for temperature-sensitive mutants that I had developed as a graduate student was shared by the Irvine labs led by H. Schneiderman and P. Bryant. Stimulated by Ernst Hadorn's work on transdetermination (HADORN 1968), I planned to exploit temperature-sensitive mutants to address two general questions: (1) What are the signals that induce cells to adopt different fates? and (2) After fate decisions are made, how are they maintained as cells divide? With the goal of developing a biochemically tractable system in that prerecombinant-DNA dark age, I looked for mutations affecting maternally encoded signals in egg cytoplasm whose disruption would upset subsequent embryogenesis in enlightening ways.

At that time, pleiotropy was a somewhat dirty word in the world of maternal effects, but I embraced it, thinking it likely that molecules involved in developmental signaling would function at multiple stages. Thus with two others at the Center for Pathobiology, I screened for interesting temperature-sensitive maternal effects among a large collection of recently isolated temperature-sensitive zy-

gotic lethals. We were surprised to discover the extent of pleiotropy: more than half of these zygotic lethals disrupted oogenesis when mutant adult females were shifted to the restrictive temperature. While pondering the difficult question of how to separate the wheat from the chaff among these mutants, I accidentally became distracted by sex.

I stumbled upon a description of the *daughterless* (*da*) gene while looking up the marker *dp* in the original "Red Book" of fly mutants (LINDSLEY and GRELL 1968), the FlyBase of its day. I read that *da*¹ mutant mothers produce sons (haplo-X progeny), but all their daughters (diplo-X progeny) die as embryos, even if they carry *da*⁺. Intrigued by such a striking maternal effect, I learned from the limited literature available that this gene was not really sex specific; *da*¹ was a hypomorphic allele of a gene whose maternal and zygotic expression was essential for both sexes. In the first of several career-altering "ahah" moments in my new life as a developmental geneticist, I imagined that *da* might be just the kind of pleiotropic gene I had been anticipating, with a sex-specific aspect to its phenotype superimposed on other aspects unrelated to sex.

This reading got me thinking about sexual dimorphism in general, and sex-specific lethality in particular, not only as interesting developmental phenomena, but also as subjects whose study seemed to have tremendous practical advantages over studies of other processes such as early pattern formation, my original interest. Although I was unsure at that point just what sex-specific embryonic lethality would reveal about development, I felt certain it would be something important—although I never imagined how quickly it would lead to specific answers to the two questions that had launched my postdoctoral research.

Although *da* had been discovered in 1954, the gene had attracted remarkably little attention. Just a few years before my epiphany, the fly geneticist L. Sandler (see the *Perspectives* article by LINDSLEY 1999) had begun

studying *da*, but with a very different motivation. Sandler believed that the maternal and zygotic effects of *da* were reflections of a single gene function acting on target genes in the heterochromatin, a function he believed was related to those of a group of genes near *da* that included *abo* (SANDLER 1977).

Following my hunch that maternal and zygotic aspects of the *da* phenotype might instead reflect very different functions, one of which was specifically essential to females, I set out to determine whether sexually mosaic progeny called gynandromorphs would survive the female-lethal *da* maternal effect. Gynandromorphs have a nearly equal mix of haplo-X and diplo-X cells, but in different orientations in different individuals. They arise by X chromosome loss in diplo-X zygotes soon after fertilization. In what tissues would X chromosome dose matter for survival? How would female cells be affected? Would male cells be affected? Would male cells rescue female cells? In the course of setting up for this simple experiment, I was pleased to discover that all aspects of the *da* mutant phenotype are temperature sensitive, a fact that I exploited in teasing apart the complex phenotype.

My hunch that *da* is pleiotropic, with disruption of one of its multiple functions causing a truly diplo-X-specific-lethal maternal effect, proved correct. Together with temperature shifts, the gynander studies showed that this maternal activity functioned within the first few hours after fertilization to disrupt the subsequent growth and differentiation of diplo-X cells even days later, wherever those cells were located and regardless of their proximity to their normally developing haplo-X neighbors (CLINE 1976). This cell-autonomous, diplo-X-specific effect on general cell growth and differentiation suggested a disruption in X chromosome dosage compensation. The fact that it also disrupted sex determination was obscured by the perverse fact that gynandromorphs never survived with abnormally differentiating diplo-X tissue in areas whose sexual phenotype could be determined unambiguously.

The greatest stroke of luck in my career helped me to identify the cause of this female-specific lethality. Since males survived this aspect of the *da* phenotype unscathed, I wondered whether there were any genetic perturbations that would allow females to survive as well. When a straightforward selection for single-hit suppressors at the restrictive temperature proved fruitless (in retrospect, only because the effort was too limited), I tried simply raising a homozygous *da* mutant stock at 18C where the viability of daughters was just high enough to maintain the line in the absence of suppression. I hoped for a slow improvement in the population sex ratio over time as weak modifiers accumulated. Far sooner than I expected, the sex ratio rose to nearly 1:1 even at higher temperatures, suggesting that all daughters as well as all sons were now surviving.

But when I tabulated the results of an outcross of

this suppressed line, I discovered that the 1:1 sex ratio reflected instead the survival of exactly half the males and half the females. A spontaneous X-linked mutation that rescued heterozygous daughters had arisen, but remained heterozygous in *da*¹ females because it killed hemizygous sons. Did those sons die simply because they lacked the wild-type allele of the suppressor gene? Answering this question required that the suppressor be mapped and its interactions with the appropriate deficiencies and duplications be determined.

That mapping effort led to the next eureka moment when it became clear that this mutation that rescued females and killed males colocalized with Muller's female-specific zygotic lethal, a mutation overlooked in the literature yet faithfully maintained by the Bowling Green Stock Center. Fortunately, chromosome rearrangements in this region that allowed me to discover that my *da* suppressor mutation was a dominant, male-specific-lethal, gain-of-function allele were available, while Muller's female-lethal mutation, which mapped just 0.007 cM away, was a loss-of-function allele that enhanced the *da* female-lethal maternal effect (CLINE 1978). Although I introduced the *Sxl*^{Male} and *Sxl*^{Female} terminology for these opposite alleles, only in the 1984 article did I finally present the full description of the formal genetic proof of allelism, a proof that generated a number of useful *Sxl*^{Mf} double mutants.

The hypothesis that *Sxl*^{Mf} and *Sxl*^{fl} were lesions in the same gene led to a simple model: expression of *Sxl*⁺ is essential to females but lethal to males. Expression is induced in females by their double dose of X chromosomes in a process that requires maternal *da*⁺. *Sxl*^{Mf} is a constitutive allele that expresses this female-specific activity regardless of X chromosome dose and hence independently of *da*⁺. Such promiscuous expression kills males. Sex-specific lethality stems from the fact that *Sxl*⁺ product imposes an overall level of X-linked gene expression appropriate only for diplo-X cells, while the level appropriate for haplo-X cells occurs only in its absence.

Soon after I joined the Princeton faculty in 1976, I turned again to gynandromorphs to reveal the developmental basis for sex-specific lethality, this time asking how haplo-X *Sxl*^{Mf}/0 mutant male tissue developed in association with diplo-X *Sxl*^{Mf}/+ female cells. Again the effect of the mutation was severe, ubiquitous, and cell autonomous. As predicted by the dosage-compensation model, mutant haplo-X tissue displayed the strong Minute phenotype expected if X chromosome gene expression was abnormally low (*Minutes* are haplo-insufficient genes, several of which are X linked). Surprisingly, however, some of the surviving affected male tissue was feminized, the first indication that sex determination and dosage compensation might be coordinately controlled by *Sxl*.

Because incompletely penetrant, gain-of-function alleles can be misleading, my excitement over the possibil-

ity of a link between these two very different aspects of sexual dimorphism was tempered until I saw the opposite effect by the loss-of-function allele: *Sxl^{fl}/Sxl^{fl}* clones generated by mitotic recombination in *Sxl^{fl}/+* females during the larval stage invariably differentiated as male. Wild-type *Sxl* product was both necessary and sufficient to impose the female pathway of sexual differentiation. Hence, at the level of the whole organism, sex-specific lethality rather than obvious sex transformations would be the hallmark of upsets in the primary events in sex determination—a major reason so little progress had been made on the sex-determination signal since 1916.

The fact that homozygous mutant clones were sex transformed even when the *Sxl⁺* allele was removed as late as the third-instar larval stage showed that *Sxl* acted relatively late in development to direct sexual differentiation, yet the involvement of maternal *da* product in the regulation of *Sxl* by X chromosome dose seemed to be very early. If *da* could be shown to affect this sex-determination function of *Sxl*, one could infer that X chromosome dose must trigger a self-propagating change in *Sxl* gene expression. With that point in mind, I ended the article reporting these results with the statement, “Of particular interest is the question of how the expression of the *Sxl* locus may be controlled by the X-chromosome/autosome balance, and whether the control is determinative in nature” (CLINE 1979, p. 274).

The first direct evidence that inappropriate *Sxl* expression upsets dosage compensation was published by LUCCHESI and SKRIPSKY (1981) in an article unfortunately plagued by confusing typos. They used the then-standard molecular assay for dosage compensation: autoradiography of labeled nascent transcripts on polytene chromosomes. I had eschewed this approach because the assay requires relatively healthy late-larval cells and is therefore biased against upsets. Moreover, the effects that one expects are of relatively low magnitude in an assay whose signal-to-noise ratio is low and for which statistical analysis is problematic. Nevertheless, an upset in dosage compensation was reported for one heteroallelic *Sxl* mutant female genotype (possibly animals who lost *Sxl⁺* activity due to a failure in maintenance rather than activation). Although no reciprocal effect of the *Sxl^{Mt}* allele on male transcription was observed, a reciprocal effect on polytene X chromosome morphology was seen.

I opted to use the approach for assaying dosage compensation that Muller had used to discover the phenomenon: measurements of adult mutant phenotypes for dosage-compensated mutant alleles. I applied the assay to triploid intersexes, XX;AAA animals that I anticipated would tolerate *Sxl*-based upsets in dosage compensation far better than diploids because the genetic imbalances predicted would be lower. Indeed, I found that triploid intersexes survived, regardless of their *Sxl* expression state, at least to the pharate adult stage (fully differentiated pupae), allowing adult morphology to be

assessed. This triploid intersex study (CLINE 1983) complemented the autoradiography study in the sense that a strong effect of *Sxl^{Mt}* on dosage compensation was seen, but the reciprocal effect of *Sxl^{fl}* was far more modest, perhaps because *Sxl⁺* might already have been off in most cells in the relevant tissues of the controls.

More significant for establishing an epigenetic aspect to the control of *Sxl⁺* was the fact that both the *da* maternal genotype and the *Sxl*zygotic genotype affected the sexual phenotype of XX;AAA animals as predicted. With their ambiguous sex-determination signal, triploid intersexes normally develop as phenotypic mosaics of male and female cells. I found that any decrease in *Sxl* activity masculinized, whether by changes in *Sxl* itself or in maternal *da* alleles, while any increase feminized. These sex effects allowed me to infer that *Sxl* control must indeed be “determinative in nature,” although I suspected that more direct evidence of this point in diploids would be needed to convince others—evidence which I presented in the 1984 article.

It is easy to underestimate today how counterintuitive the argument once seemed for an epigenetic aspect to sex determination and how the overly broad use of the term “sex determination” had blurred important potential distinctions among events that initially set sexual fate, events that then maintain it in growing cells, and events that finally direct morphogenesis in terminally differentiating cells. Prior to the work with *Sxl*, the standard developmental sense of “determination” in *Drosophila* had always involved the paradigm of heritable developmental fate differences maintained epigenetically between genetically identical cells. Since male and female *Drosophila* cells were genetically different with respect to the key variable, what could sex determination possibly have to contribute regarding epigenetic processes of cell fate restriction?

Years earlier, STERN (1966) had shown that the mosaic sexual phenotype of triploid intersexes reflected genetically identical cells displaying opposite sexual phenotypes; however, without an understanding of *Sxl* and information on when in development such mosaicism developed, the implications of this intersexuality were unclear. Equally unclear were implications of another qualitatively different class of intersex: “true intersexes,” in which even individual cells displayed a sexually intermediate phenotype. The significance of the distinction between these two classes of intersexes, and of the fact that both classes can be generated by mutations in *Sxl*, were important elements in the 1984 article, although the points are discussed more explicitly in the reviews that soon followed (*e.g.*, MAINE *et al.* 1985).

It might seem more straightforward to deduce that the activity state of *Sxl* is maintained independently of the X chromosome dose signal that initiated it, from experiments in which the X chromosome dose was changed at various times in development, than from experiments leaving the X chromosome dose unchanged. This direct

approach was in fact attempted by SANCHEZ and NOTHIGER (1983). But while their experiments were suggestive, their approach was fatally flawed, not only by an experimental design that did not include convincing evidence that the X chromosome dose had in fact been changed, but also by the fact that the cells whose X chromosome dose was thought to have changed simply died, disappearing before their sexual phenotype could be assessed. Hence, the possibility could not be excluded that the activity state of *Sxl* could be reversed, but simply not fast enough to avoid a transient growth handicap that would induce nonhandicapped neighbors to exclude them. Moreover, data (SANCHEZ and NOTHIGER 1983, Table 4) conflicting with their hypothesis regarding timing were ignored.

I had expected that an experiment that I began in 1979 would unambiguously demonstrate the epigenetic effect of *da* on *Sxl*'s sex-determination function in diploids. This experiment failed, but its failure led to the hypothesis—which experiments in the 1984 article substantiated—that *Sxl* had a female-specific autoregulatory activity that could act *in trans* to bypass the normal *Sxl* activation process, an autoregulatory activity that might normally serve to maintain the active state triggered by the X chromosome dose. I had thought that the *da* maternal effect would block activation of the *Sxl*⁺ allele present in *Sxl*^{Mt}/*Sxl*⁺ diploid daughters that had been rescued by *Sxl*^{Mt} and hence that *Sxl*⁺/*Sxl*⁺ clones induced in these daughters by mitotic recombination would survive and differentiate as male, just like the *Sxl*^f/*Sxl*^f clones induced in *Sxl*^f/*Sxl*⁺ females. To my chagrin, such clones always differentiated as female. Clearly, the *Sxl*⁺ allele was active.

I guessed that *Sxl*⁺ activation in this case had been induced by wild-type *Sxl* product generated from the constitutive allele *Sxl*^{Mt} *in trans*. To test this hypothesis, I had to find a situation in diploid females in which activation of *Sxl*⁺ was blocked by the *da* maternal effect and transactivation did *not* occur, yet the cells still survived to display a masculinized phenotype that would unambiguously indicate that block. Moreover, if diplo-X cells survived with what I inferred to be a silent *Sxl*⁺ allele, I should be able to remove that allele without further effect. The 1984 article described such situations, thereby establishing the reality of *Sxl* autoregulation.

The successful approach was based on the expectation that the death of diplo-X cells lacking *Sxl*⁺ activity was due to inappropriate hyperactivation of their X-linked genes, and therefore that if there were some way to block that hyperactivation, diplo-X cells with epigenetically silenced *Sxl*⁺ alleles should survive to reveal their masculinization. BELOTE and LUCCHESI (1980) reported the first evidence that a member of the set of genes known as *male-specific lethals* (*msl*'s) was required for the transcriptional hyperactivation associated with male dosage compensation. Would mutations in these *msl*'s suppress *Sxl*-based female lethality by blocking inappropriate

hyperactivation? The confusing initial answer was “possibly yes and possibly no”: “yes” at the level of individual cells, but “no” at the level of the whole organism. Only when impairment of *Sxl* function was sufficiently mild to allow even *msl*⁺ females to survive would mutations in the *msl*'s cause a mosaic intersex phenotype. But did this intersexuality really reflect cell rescue, or instead might the *msl* mutations simply reduce the effectiveness of *Sxl*⁺ product in directing female differentiation? Since mutations in the *msl*'s by themselves did not affect sexual phenotype, any such reduction in *Sxl* effectiveness would have to be so mild that it was apparent only when *Sxl*⁺ levels were also reduced.

Three key points emphasized in the 1984 article supported the view that the *msl* mutations rescue functionally *Sxl*[−] cells and excluded the alternative: First, *msl* mutations suppressed developmental damage that had clearly indicated sporadic cell death among the surviving daughters of *da* mutant mothers. Sporadic masculinization was seen in its place. Second, at the cellular level, sporadic masculinization was always of the “all-or-none” type, never the incomplete (“true” intersex) type that one would expect from a simple reduction in *Sxl*⁺ effectiveness. Finally, the degree of interspersion of male and female cells showed that the critical events generating these mosaic intersexes occurred as soon as the imaginal disc primordia were formed, not later when the discs terminally differentiated.

The 1984 article dealt at some length with the question of why *msl* mutations rescued functionally *Sxl*[−] cells but not whole animals. The *msl* genes simply might not control as many aspects of dosage compensation as *Sxl* does, with regard to developmental time, tissue type, or gene target. One would expect rescue of imaginal disc cells to be easier than rescue of the whole organism if suppression of the dosage-compensation upset was incomplete, particularly if it were more incomplete earlier in development.

GERGEN (1987) confirmed the key element in this hypothesis by showing that *runt* dosage compensation at the blastoderm stage is controlled by *Sxl* but not by the *msl*'s. Subsequently, FRANKE *et al.* (1996) and RASTELLI *et al.* (1995) showed that the *msl*'s are unlikely to control any dosage compensation at the blastoderm stage [although one would not guess that from the FRANKE *et al.* (1996) title]. KELLEY *et al.* (1995) presented a bioinformatic argument that X-linked genes are far more likely than autosomal genes to have multiple potential *Sxl*-binding sites in their 3'-UTRs (20 X-linked genes including *runt* vs. only two autosomal: *msl-1* and *msl-2!*). They proposed that these sites might reflect an *msl*-independent post-transcriptional dosage-compensation mechanism directly mediated by *Sxl* in females. J. KAMINKER, G. RUBIN and T. CLINE (unpublished results) extended this approach, using data on 2235 X-linked and 11,887 autosomal 3'-UTRs, respectively. Of the 84 potential *Sxl* target genes identified, 70 were on the X, including

the dosage-compensation gene *mof*. Moreover, *mvl-3* was added to *mvl-1* and *mvl-2* among only 14 potential autosomal targets. A significant post-transcriptional contribution to dosage compensation would account for the often overlooked fact that transcriptional hyperactivation of X-linked genes in males relative to females is only 1.4 times overall, not 2 times (HOLMQUIST 1972).

The argument that *mvl* mutations rescue cells in which *Sxl*⁺ has been epigenetically silenced by *da* was clinched in the 1984 article by experiments exploiting *Sxl*^{7,MI}, a double-mutant allele recovered as a male-viable derivative of *Sxl*^{MI}. The article also provided the strongest evidence for *Sxl* positive autoregulation. The *Sxl*^{MI} lesion allows *Sxl*^{7,MI} to produce *Sxl*⁷ mutant protein even in daughters of *da* mutant mothers under the most nonpermissive conditions. *Sxl*⁷ protein cannot elicit female differentiation, but it does still have some dosage-compensation and autoregulatory activity. Since *Sxl*^{7,MI} cannot feminize by itself, the sexual phenotype of *Sxl*^{7,MI}/*Sxl*⁺ cells reveals the activity state of *Sxl*⁺.

One to three percent of *Sxl*^{7,MI}/*Sxl*⁺ daughters survived the *da* maternal effect under conditions invariably lethal to their *Sxl*⁺/*Sxl*⁺ sisters. The sexual phenotype of these survivors showed they had activated *Sxl*⁺ in all nongonadal cells. With an additional copy of *Sxl*^{7,MI}, 49% of the daughters survived, again with *Sxl*⁺ activated in all nongonadal tissues. In contrast, doubling the dose of *Sxl*⁺ had relatively little effect, as expected if *Sxl*⁺ alleles were being silenced by the *da* maternal effect, and their activation by *Sxl*^{7,MI} was rate limiting.

This dose effect of *Sxl*^{7,MI} had to be due to an increase in the activation of *Sxl*⁺ by *Sxl*^{7,MI}, since when *Sxl*^{7,MI}/*Sxl*⁺ daughters were also mutant for *mvl*'s, 70% survived, but nearly all had a mosaic intersex phenotype signaling their failure to have activated *Sxl*⁺ in all nongonadal cells. For the first time, *mvl* mutations rescued whole animals, not just cells. Most importantly, *mvl* mutations did *not* increase the recovery or alter the phenotype of daughters who would have survived without *mvl* mutations—those that had transactivated *Sxl*⁺ in all nongonadal cells.

Some *Sxl*^{7,MI}/*Sxl*⁺ daughters mutant for *mvl*'s were entirely male, indicating that they had survived the *da* maternal effect without activating *Sxl*⁺ in any adult precursor cells. This result suggested that *mvl* mutations might be able to rescue *Sxl*^{7,MI}/*Df*(*Sxl*⁺) females, a genotype not otherwise viable. This prediction of the “epigenetically silenced *Sxl*⁺ allele” hypothesis was validated.

The degree of interspersion of male and female cells in the forelegs of *mvl*-rescued females showed that if activation of *Sxl*⁺ by autoregulation was going to occur, it occurred only very early. Preliminary results of mitotic recombination experiments were mentioned, confirming that the *Sxl*⁺ activity state established so early was heritably maintained thereafter. But the question of whether continued *Sxl* autoregulation maintained female fate (with male fate being the default state) was

answered definitively in the affirmative only years later with construction of a *Sxl*⁺ transgene whose expression could be manipulated (BELL *et al.* 1991).

The 1984 article described a variety of intriguing effects on the development of the ovary that we are still working to understand, including the inability of *Sxl*^{7,MI} to transactivate *Sxl*⁺ alleles in the gonadal soma. The 1984 study came tantalizingly close to revealing the important role of the gonadal mesoderm in feminizing diplo-X germ cells, but it lacked the key *Sxl*^{7,MI} pole-cell transplant experiment required for such a conclusion.

Seven years after the 1984 article demonstrated positive autoregulation as a mechanism by which *Sxl* might maintain the female fate decision in dividing cells, we described the molecular basis for this phenomenon (BELL *et al.* 1991): Transcripts generated from the maintenance promoter of *Sxl*, a promoter that is active in all cells of both sexes, can be processed into mRNA that encodes full-length *Sxl* protein only if full-length *Sxl* protein is already present. Hence, the active (female) state is maintained by a positive-feedback loop on *Sxl* pre-mRNA splicing, while the inactive (male) state is maintained by default. Of course, the question of the origin of the initial *Sxl* protein that engages this feedback loop in females remained.

Earlier genetic analysis had indicated that the mechanism by which the female state of *Sxl* expression was initiated by the X chromosome dose signal was likely to be very different from the mechanism by which it was subsequently maintained independently of that signal, since these two aspects of regulation could be disrupted separately by different mutations in *Sxl* (MAINE *et al.* 1985). In 1992, we demonstrated that two different *Sxl* promoters were involved (KEYES *et al.* 1992). Transient activation of the establishment promoter in females in response to their double dose of X chromosomes is able to generate a burst of *Sxl* protein that subsequently will engage the autoregulatory loop for maintenance promoter transcripts because productive processing of transcripts from the establishment promoter does not require *Sxl* protein. The labels “establishment” and “maintenance” for the two *Sxl* promoters emphasize the striking formal parallels between initiation and maintenance of the female pathway choice in *Drosophila* and the lysogenic developmental pathway paradigm for phage λ , key elements of which are the repressor establishment and repressor maintenance promoters (see PTASHNE 2004).

Undeniable analogies exist between the strategy that the fruit fly uses for sex determination and strategies used by a variety of organisms for initiating and maintaining other developmental fate decisions, many of which are known to involve binary switch genes like *Sxl*. Nevertheless, perhaps the greatest irony of the *Sxl* autoregulation story is that even now, so many years after the 1984 report, no other case of a developmental fate decision being maintained by positive feedback on

pre-mRNA splicing seems to have emerged. Nature—or at least those who study Nature—would seem to be missing a good bet!

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