

ing RNAs that act as riboregulators (53) and determine the degree to which they contribute to epigenetic regulation of gene expression in diverse organisms.

### References and Notes

- M. Fagard, H. Vaucheret, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **51**, 167 (2000).
- A. Fire et al., *Nature* **391**, 806 (1998).
- M. A. Matzke, A. J. M. Matzke, G. Pruss, V. Vance, *Curr. Opin. Genet. Dev.* **11**, 221 (2001).
- S. M. Hammond, A. A. Caudy, G. J. Hannon, *Nat. Rev. Genet.* **2**, 110 (2001).
- V. B. Vance, H. Vaucheret, *Science*, **292**, 2277 (2001).
- T. Tuschl, *ChemBiochem* **2**, 239 (2001).
- R. W. Carthew, *Curr. Opin. Cell Biol.* **13**, 244 (2001).
- C. Cogoni, G. Macino, *Curr. Opin. Genet. Dev.* **10**, 638 (2000).
- P. Sharp, *Genes Dev.* **15**, 485 (2001).
- P. Plasterk, R. Ketting, *Curr. Opin. Genet. Dev.* **10**, 562 (2000).
- T. Tuschl, P. D. Zamore, R. Lehmann, D. P. Bartel, P. A. Sharp, *Genes Dev.* **13**, 3191 (1999).
- S. M. Hammond, E. Bernstein, D. Beach, G. J. Hannon, *Nature* **404**, 293 (2000).
- P. D. Zamore, T. Tuschl, P. A. Sharp, D. P. Bartel, *Cell* **101**, 25 (2000).
- S. M. Elbashir, W. Lendeckel, T. Tuschl, *Genes Dev.* **15**, 188 (2001).
- E. Bernstein, A. A. Caudy, S. M. Hammond, G. J. Hannon, *Nature* **409**, 363 (2001).
- A. Hamilton, D. Baulcombe, *Science* **286**, 950 (1999).
- S. M. Hammond, S. Boettcher, A. Caudy, R. Kobayashi, G. J. Hannon, *Science*, in press.
- L. Cerutti, N. Mian, A. Bateman, *Trends Biochem. Sci.* **25**, 481 (2000).
- D. Wu-Scharf, B.-r. Jeong, C. Zhang, H. Cerutti, *Science* **290**, 1159 (2000).
- A. Mallory et al., *Plant Cell* **13**, 571 (2001).
- C. Llave, K. D. Kasschau, J. C. Carrington, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 13401 (2000).
- M. F. Mette, A. Matzke, M. Matzke, *Curr. Biol.*, **11**, 1119 (2001).
- I. Papaefthimiou et al., *Nucleic Acids Res.* **29**, 2395 (2001).
- S. E. Jacobsen, M. P. Running, E. M. Meyerowitz, *Development* **126**, 5231 (1999).
- O. Voinnet, C. Lederer, D. Baulcombe, *Cell* **103**, 157 (2000).
- M. Wassenegger, *Plant Mol. Biol.* **43**, 203 (2000).
- M. F. Mette, W. Aufsatz, J. van der Winden, M. A. Matzke, A. J. M. Matzke, *EMBO J.* **19**, 5194 (2000).
- T. Sijen et al., *Curr. Biol.* **11**, 436 (2001).
- L. Jones, F. Ratcliff, D. Baulcombe, *Curr. Biol.* **11**, 747 (2001).
- J. B. Morel, P. Mourrain, C. Béclin, H. Vaucheret, *Curr. Biol.* **10**, 1591 (2000).
- X. Cao et al., *Proc. Natl. Acad. Sci. U.S.A.* **97**, 4979 (2000).
- M.-B. Wang, S. V. Wesley, E. J. Finnegan, N. A. Smith, P. M. Waterhouse, *RNA* **7**, 16 (2001).
- S. S. Smith, J. L. Kan, D. J. Baker, B. E. Kaplan, P. Dembeck, *J. Mol. Biol.* **217**, 39 (1991).
- A. M. Lindroth et al., *Science* **292**, 2077 (2001).
- A. Akhtar, D. Zink, P. B. Becker, *Nature* **407**, 405 (2000).
- J. T. Lee, *Cell* **103**, 17 (2000).
- A. Pannuti, J. C. Lucchesi, *Curr. Opin. Genet. Dev.* **10**, 644 (2000).
- W. Reik, J. Walter, *Nat. Rev. Genet.* **2**, 21 (2001).
- D. L. J. Lafontaine, D. Tollervey, *Trends Biochem. Sci.* **23**, 383 (1998).
- T. H. Bestor, *Human Mol. Genet.* **9**, 2395 (2000).
- E. J. Finnegan, K. A. Kovac, *Plant Mol. Biol.* **43**, 189 (2000).
- T. Singer, C. Yordan, R. Martienssen, *Genes Dev.* **15**, 591 (2001).
- A. Miura et al., *Nature* **411**, 212 (2001).
- H. Hirochika, H. Okamoto, T. Kakutani, *Plant Cell* **12**, 357 (2000).
- M. A. Matzke, M. F. Mette, A. J. M. Matzke, *Plant Mol. Biol.* **43**, 401 (2000).
- A. Pasquinelli et al., *Nature* **408**, 86 (2000).
- G. Hutvagner et al., *Science* **293**, 834 (2001).
- A. Grishok et al., *Cell* **106**, 23 (2001).
- E. H. Davidson, R. J. Britten, *Science* **204**, 1051 (1979).
- R. Barstead, *Curr. Opin. Chem. Biol.* **5**, 63 (2001).
- S. Elbashir et al., *Nature* **411**, 494 (2001).
- J. A. Doudna, *Nature Struct. Biol. Suppl.* **7**, 954 (2000).
- V. A. Erdmann et al., *Nucleic Acids Res.* **29**, 189 (2001).
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### VIEWPOINT

# Epigenetic Aspects of X-Chromosome Dosage Compensation

Yongkyu Park and Mitzi I. Kuroda

The X chromosomes of mammals and fruit flies exhibit unusual properties that have evolved to deal with the different dosages of X-linked genes in males (XY) and females (XX). The X chromosome dosage-compensation mechanisms discovered in these species are evolutionarily unrelated, but exhibit surprising parallels in their regulatory strategies. These features include the importance of noncoding RNAs, and epigenetic spreading of chromatin-modifying activities.

Sex chromosomes have posed a fascinating puzzle for biologists. The dissimilar organization, gene content, and regulation of the X and Y chromosomes are thought to reflect selective forces acting on original pairs of identical chromosomes (1–3). The result in many organisms is a male-specific Y chromosome that has lost most of its original genetic content, and a difference in dosage of the X chromosome in males (XY) and females (XX).

The processes that animals use to respond to differences in X-chromosome dosage have been most intensively studied in three very different groups of organisms: (i) mammals, including marsupials; (ii) the nematode *Caenorhabditis elegans*; and (iii) the fruit fly, *Drosophila melanogaster*.

The dosage-compensation mechanisms in these species differ dramatically: eutherian (placental) mammals randomly inactivate one of their two X chromosomes in females to form a Barr body (4); female marsupials display non-random X inactivation (5); XX hermaphrodite nematodes halve the expression from each X (6); and male *Drosophila* increase the transcription of their single X approximately twofold (7, 8). In each case however, the regulation of chromatin composition and structure in one sex is central to the dosage-compensation mechanism. The striking result is sex- and chromosome-specific localization of activities that acetylate and deacetylate histones, methylate DNA, condense chromosomes, or otherwise modify chromatin architecture. The chromosome-specific mechanisms that have been in-

vestigated are either very unusual, or are simply dramatic examples of regulation that might be much more common than currently recognized.

What are these unusual aspects of X-chromosome regulation? We focus on mammalian and fruit fly dosage compensation to illustrate surprising parallels in their distinct regulatory strategies. The first common aspect is that in each case, chromatin-modifying activities spread long distances in cis from specific initiation sites on the X chromosome (9–11). The second parallel is that these initiation sites produce a new class of untranslated, chromosome-associated RNAs (12–15).

In mammals, the *Xist* RNA originates from the X inactivation center (*Xic*), “coats” one chromosome in cis, and is required for X inactivation (12, 13, 16, 17). The *Tsix* gene is transcribed in an antisense orientation to *Xist* and regulates the choice of which X chromosome remains active (18). *Xist* transgenes inserted on autosomes can cause DNA methylation, histone hypoacetylation, late replication, accumulation of a variant histone (macroH2A), and transcriptional inactivation of distantly linked genes, all characteristics of the endogenous inactive X chromosome (9, 10, 19) (Fig. 1).

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In flies, at least two dissimilar *roX* RNAs originate from separate genes on the *Drosophila* X chromosome, associate with the male X chromosome along its length, and have overlapping genetic functions in X hypertranscription (14, 15, 20). Just as *Xist* transgenes can exert effects on flanking autosomal chromatin in mammals, *roX* transgenes inserted on autosomes can cause site-specific histone acetylation of distantly linked genes that are not normally targets of *Drosophila* dosage compensation (11, 21) (Fig. 1).

The regulation of X inactivation in mammals is a classic example of "epigenetics." In human and mouse, two virtually identical X chromosomes are present in each female nucleus, yet only one is inactivated, expressing the *Xist* RNA that coats the chromosome strictly in cis (4). The other X escapes *Xist* silencing and remains active. The two opposing activity states of the X chromosomes are clonally inherited throughout the life of the female. The initial choice of which X to inactivate is made early in embryogenesis, just before implantation of the blastocyst. In those cells that will go on to form the embryo proper, the choice of which X to inactivate is random, whereas in cells that will form the extraembryonic tissues (e.g., placenta and yolk sac), it is always the paternal X that is chosen for inactivation.

Current models highlight dynamic roles for both *Xist* and its antisense counterpart, *Tsix*. *Tsix*, like *Xist*, is located in the genetically defined X inactivation center, and the activity of both genes has been studied in mice and in XX embryonic stem cell lines, where X inactivation can be induced during differentiation in cell culture. Recent studies have shown not only that *Xist* is required for X inactivation in cis (16, 17), but also that a high level of *Xist* expression may be sufficient to initiate the inactivation process in the absence of the rest of the *Xic* (22). In contrast, *Tsix* influences the choice of which X remains active, because a deletion of the *Tsix* promoter leads to constitutive inactivation of the mutant X chromosome (18). A linked region beyond the *Tsix* promoter also influences X-chromosome choice (23, 24). Interestingly, *Tsix* regulation is only manifested in XX animals, because male mice carrying a *Tsix* deletion retain an active X chromosome (18). This could be explained by the clear requirement for counting two or more X chromosomes, or X inactivation centers, before X inactivation can proceed (4). Counting is independent of sex determination per se; therefore, XX females or XXY males each produce one inactive X chromosome, whereas XY males or XO females do not. Understanding the molecular basis for counting X chromosomes could prove to be a difficult problem if multiple, noncontiguous segments of the X inactivation center are required.

Another striking epigenetic aspect of *Xist* function is that the *Xist* locus is dispensable for

maintenance of the inactive state (25, 26). Surprisingly, once the chromatin architecture of an inactive X chromosome is established, it can be inherited without the continuous presence of *Xist*. Normally, however, *Xist* RNA does coat the inactive X throughout the lifetime of a female, and sensitive assays have revealed that continued presence of *Xist* RNA acts synergistically with DNA methylation and histone hypoacetylation to accomplish high-fidelity inheritance of the inactive state (27).

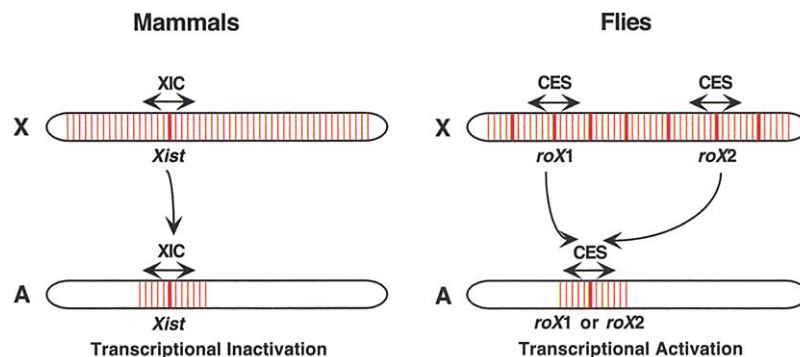
The random X inactivation exhibited in eutherian mammals is thought to have evolved from a more ancient imprinting mechanism (28). This nonrandom mechanism, in which the paternal X chromosome is always inactivated, is still seen in marsupials and in extraembryonic tissues during early mouse development. In mouse, the *Tsix* promoter is essential for protecting the maternal X from inactivation in extraembryonic tissues (29, 30). Progeny of either sex who receive a *Tsix* deletion from their mother exhibit severely reduced viability due to a failure to maintain any active X chromosomes in their extraembryonic tissues. At present, whether *Tsix* exerts this key regulation through the act of antisense transcription, through an antisense RNA molecule, or simply as a DNA element is not known (29–31). As yet, no *Xist* or *Tsix* homologs have been discovered in marsupials, although histone hypoacetylation of the inactive X is a conserved feature (32).

In contrast to X inactivation in mammals, the regulation of X hypertranscription in flies is not a classic example of "epigenetics" but may use related mechanisms for the establishment and propagation of chromatin organization. The male X is hypertranscribed through the action of a large complex containing at least two *roX* RNAs and five polypeptides, collectively termed the Male-Specific Lethal (MSL) proteins (7, 8). Males require each of these proteins for viability and die when any of these are absent. The MSL complex is required for specific histone H4 acetylation at Lys-16 on the

male X chromosome, which is likely to be a key component of the up-regulation mechanism (33–36). Another chromatin-modifying factor, the histone H3 kinase JIL-1, is also implicated in dosage compensation (37). Unlike the MSL proteins, JIL-1 also plays an essential role in both sexes (38).

The decision to carry out dosage compensation or not is controlled by a series of sex-specific binary genetic switches (39). Early in embryonic development, *Sex lethal* (*Sxl*) is turned on in response to the presence of two X chromosomes. *Sxl*, in turn, blocks translation of *msl2* RNA in females. MSL complexes are not found in the absence of MSL2, so females do not use dosage compensation to regulate their two X chromosomes.

Epigenetic aspects of fruit fly dosage compensation become evident when considering how the MSL complex distinguishes the X chromosome from the autosomes. The original expectation was that the complex would function as a conventional trans-acting factor, directly binding some simple sequence repeated hundreds of times along the length of the X. Unexpectedly, the MSL complex has instead been shown to have the ability to spread in cis, apparently moving long distances from defined "chromatin entry sites" into flanking sequences (11). About 35 chromatin entry sites are thought to nucleate spreading of MSL complexes along the length of the X, and two of these are the *roX1* and *roX2* genes (11, 21). Spreading of MSL complexes, including *roX* RNAs, is seen ectopically on autosomes when *roX* loci are inserted as transgenes. Although it is still not evident what sequence or structure defines a chromatin entry site, surprisingly, *roX1* RNA transcription from an autosomal *roX1* transgene does not appear to be essential for attracting MSL complexes and nucleating spreading (40). Rather, deletion analysis has identified a 200–base pair segment of *roX1* DNA that is sufficient for providing an entry site without itself



**Fig. 1.** The spreading in cis of chromatin-remodeling activities from *Xist* and *roX* transgenes. In mammals, *Xist* RNA is transcribed from the X inactivation center (XIC) and spreads to inactivate the X chromosome in cis. *Xist* transgenes inserted on autosomes also cause the spreading of transcriptional inactivation in cis. In flies, there are about 35 chromatin entry sites (CES) on the X chromosome, including *roX1* and *roX2*. Unlike the XIC in mammals, the entry sites in flies regulate twofold transcriptional activation. However, like *Xist* transgenes, *roX1* and *roX2* transgenes inserted on autosomes also show spreading in cis of chromatin remodeling.

producing a detectable RNA product. This does not, however, preclude a role for *roX* RNAs in the spreading process, because MSL complexes attracted to such a minimal *roX1* DNA site bring *roX* RNAs (expressed from their endogenous locations) as components of the complex. Interestingly, the histone acetylation activity of the complex is required for its ability to spread from chromatin entry sites into flanking sequences (41).

Several recent developments provide new insights into the epigenetic nature of dosage compensation in *Drosophila*. First, in addition to site-specific histone H4 acetylation (33, 34), histone H3 phosphorylation and acetylation have been implicated in X-chromosome hypertranscription through the analysis of the JIL-1 histone H3 kinase (38). JIL-1 is concentrated on the male X chromosome where it phosphorylates histone H3 at Ser-10. In mammalian cells, phosphorylation of histone H3 is coupled to H3 acetylation during growth factor-triggered gene activation (42, 43). This coupled relationship appears also to hold true for the up-regulated male X chromosome in flies, because antibodies that specifically recognize only phospho-acetyl H3 also preferentially immunostain the male X during interphase (38). These discoveries highlight the likelihood that additional chromatin modifications will be revealed on dosage-compensated X chromosomes in flies and in mammals as new site- and modification-specific antibodies continue to be developed.

A completely unexpected recent development regarding chromosome-wide regulation in fruit flies involves an autosome-specific protein, POF (Painting of Fourth), discovered in *D. melanogaster* on the tiny fourth chromosome (44) (Fig. 2). POF is a putative RNA binding protein with an RRM domain. Although expressed at a higher level in males than in females, it binds the fourth chromosome in both sexes. Through translocation studies, it appears that painting of the chromosome may use a

spreading mechanism, because the distal tip of the chromosome is not bound by the POF protein unless linked in cis to the proximal region of the fourth chromosome.

Autosomes are not expected to have chromosome-specific transcriptional regulation, but Larsson *et al.* (44) drew several disparate observations together to examine a possible evolutionary relationship between the fourth chromosome and the X. A key discovery came from examination of *Drosophila busckii*, a species in which genes found on the *D. melanogaster* fourth chromosome are instead inserted at the base of the X (45). When stained with antibodies to POF, the entire X chromosome of *D. busckii* is seen to be "painted" by the POF protein (44) (Fig. 2). This result is male-specific, suggesting that POF is participating in dosage compensation. In contrast, antibodies to the *D. melanogaster* MSL proteins, which cross-react with many *Drosophila* species previously tested (46, 47), failed to stain the *D. busckii* X chromosome (44). Although this may simply mean that the *D. busckii* MSL proteins have diverged considerably, could it be possible that an alternative system of dosage compensation is used even within the same genus? POF is a putative RNA binding protein, so it will be interesting to see if untranslated RNAs analogous to *roX* RNAs may be involved in the proposed spreading of POF along chromosomes.

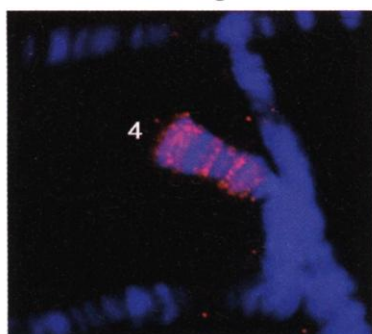
The epigenetic aspects of X-chromosome dosage compensation in mammals and fruit flies suggest a potential mechanism for rapid evolutionary change in the regulation of whole chromosomes. Such rapid change could be facilitated through the selective localization of chromatin-re modeling machines that can then spread long distances in cis from their original sites of entry. The mechanistic role of RNAs as key components in such complex-

es is not yet known. Although these examples highlight whole chromosomes as recipients of such regulation, it remains to be seen if the general principles discovered may also apply to smaller chromosome domains during cell specialization, differentiation, and development.

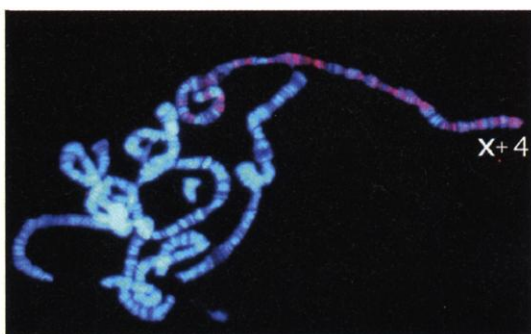
## References

1. B. Charlesworth, *Curr. Biol.* **6**, 149 (1996).
2. W. R. Rice, *Bioscience* **46**, 331 (1996).
3. B. T. Lahn, D. C. Page, *Science* **286**, 964 (1999).
4. P. Avner, E. Heard, *Nat. Rev. Genet.* **2**, 59 (2000).
5. D. W. Cooper, P. G. Johnston, J. M. Watson, J. A. M. Graves, *Semin. Dev. Biol.* **4**, 117 (1993).
6. B. J. Meyer, *Trends Genet.* **16**, 247 (2000).
7. R. L. Kelley, M. I. Kuroda, *Curr. Opin. Genet. Dev.* **10**, 555 (2000).
8. A. Pannuti, J. C. Lucchesi, *Curr. Opin. Genet. Dev.* **10**, 644 (2000).
9. J. T. Lee, R. Jaenisch, *Nature* **386**, 275 (1997).
10. L. B. K. Herzing, J. T. Romer, J. M. Horn, A. Ashworth, *Nature* **386**, 272 (1997).
11. R. L. Kelley *et al.*, *Cell* **98**, 513 (1999).
12. C. J. Brown *et al.*, *Nature* **349**, 38 (1991).
13. C. M. Clemson, J. A. McNeil, H. F. Willard, J. B. Lawrence, *J. Cell Biol.* **132**, 259 (1996).
14. H. Amrein, R. Axel, *Cell* **88**, 459 (1997).
15. V. H. Meller, K. H. Wu, G. Roman, M. I. Kuroda, R. L. Davis, *Cell* **88**, 445 (1997).
16. G. D. Penny, G. F. Kay, S. A. Sheardown, S. Rastan, N. Brockdorff, *Nature* **379**, 131 (1996).
17. Y. Marahrens, B. Panning, J. Dausman, W. Strauss, R. Jaenisch, *Genes Dev.* **11**, 156 (1997).
18. J. T. Lee, N. Lu, *Cell* **99**, 47 (1999).
19. T. P. Rasmussen, A. Wutz, J. R. Pehrson, R. Jaenisch, *Chromosoma*, published online 20 July 2001 (DOI 10.1007/s004120100158).
20. A. Franke, B. S. Baker, *Mol. Cell* **4**, 117 (1999).
21. V. H. Meller *et al.*, *Curr. Biol.* **10**, 136 (2000).
22. A. Wutz, R. Jaenisch, *Mol. Cell* **5**, 695 (2000).
23. P. Clerc, P. Avner, *Nature Genet.* **19**, 249 (1998).
24. C. Morey, D. Arnaud, P. Avner, P. Clerc, *Hum. Mol. Genet.* **10**, 1403 (2001).
25. C. J. Brown, H. F. Willard, *Nature* **368**, 154 (1994).
26. G. Csankovszki, B. Panning, B. Bates, J. R. Pehrson, R. Jaenisch, *Nature Genet.* **22**, 323 (1999).
27. G. Csankovszki, A. Nagy, R. Jaenisch, *J. Cell Biol.* **153**, 773 (2001).
28. D. Solter, G. Wei, *Genes Dev.* **11**, 153 (1997).
29. J. T. Lee, *Cell* **103**, 17 (2000).
30. T. Sado, Z. Wang, H. Sasaki, E. Li, *Development* **128**, 1275 (2001).
31. E. Debrand, C. Chureau, D. Arnaud, P. Avner, E. Heard, *Mol. Cell Biol.* **19**, 8513 (1999).
32. M. J. Wakefield, A. M. Keohane, B. M. Turner, J. A. Graves, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 9665 (1997).
33. B. M. Turner, A. J. Birley, J. Lavender, *Cell* **69**, 375 (1992).
34. A. Hilfiker, D. Hilfiker-Kleiner, A. Pannuti, J. C. Lucchesi, *EMBO J.* **16**, 2054 (1997).
35. E. R. Smith *et al.*, *Mol. Cell Biol.* **20**, 312 (2000).
36. A. Akhtar, P. B. Becker, *Mol. Cell* **5**, 367 (2000).
37. Y. Jin, Y. Wang, J. Johansen, K. M. Johansen, *J. Cell Biol.* **149**, 1005 (2000).
38. Y. Wang, W. Zhang, Y. Jin, J. Johansen, K. M. Johansen, *Cell* **105**, 433 (2001).
39. T. W. Cline, B. J. Meyer, *Annu. Rev. Genet.* **30**, 637 (1996).
40. Y. Kageyama *et al.*, *EMBO J.* **20**, 2236 (2001).
41. W. Gu, X. Wei, A. Pannuti, J. C. Lucchesi, *EMBO J.* **19**, 5202 (2000).
42. P. Cheung *et al.*, *Mol. Cell* **5**, 905 (2000).
43. A. L. Clayton, S. Rose, M. J. Barratt, L. C. Mahadevan, *EMBO J.* **19**, 3714 (2000).
44. J. Larsson, J. D. Chen, V. Rasheva, A. Rasmuson-Lestander, V. Pirrotta, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 6273 (2001).
45. J. Krivshenko, *Genetics* **44**, 1027 (1959).
46. J. R. Bone, M. I. Kuroda, *Genetics* **144**, 705 (1996).
47. I. Marin, A. Franke, G. J. Bashaw, B. S. Baker, *Nature* **383**, 1/60 (1996).

## *D. melanogaster*



## *D. busckii*



**Fig. 2.** Surprising chromosome-specific localization of the POF protein in *D. melanogaster* and *D. busckii*. (Left) POF is specifically localized to the fourth chromosome in *D. melanogaster*. (Right) POF is associated with the X chromosome of *D. busckii*, which is a composite of the X and fourth chromosomes found in *D. melanogaster*. Because X chromosome immunostaining in *D. busckii* is male-specific, POF may be part of a novel X chromosome dosage-compensation system. [Adapted from (44)]