Sex, flies and neuronal fates

Anuranjan Anand and H. Sharat Chandra

Sex in *Drosophila melanogaster* is determined by the ratio of the number of X chromosomes to the number of sets of autosomes. This parameter, the X : A ratio, acts to set the functional state of a major regulatory gene, *Sex-lethal* (*Sxl*). An X : A ratio of 1 results in activation of *Sxl* and this is essential for female development. When this ratio is 0 5, *Sxl* is not activated and male development ensues. The X : A ratio is thought to be assessed by means of discrete chromosomal sites called 'counting elements'. 'Numerator' elements are those counting elements that are located on the X chromosome. By increasing the probability of activation of *Sxl*, these numerator elements behave as feminizing genes. Three such genes are known: *sisterless-a* (*sis-a*), *sisterless-b* (*sis-b*) and *runt*. They are functionally related because a defect in one can be partly offset by a duplication of either of the other two. These genes cause female lethality and show dose-dependent, reciprocal, and sex-specific interactions among themselves and with *Sxl*, the switch gene[1–4]. These numerator genes code for transcription factors. *sis-a* encodes a leucine zipper protein[5] and *sis-b*, a protein with a basic helix–loop–helix (bHLH) DNA-binding domain and a protein–protein dimerization domain[6,7]. *runt*, which encodes a nuclear protein implicated in transcriptional regulation, ensures the uniform expression of *Sxl* throughout the embryo. *Sxl* activation requires maternal inputs as well. The gene *daughterless* (*da*), which codes for a maternally expressed basic helix–loop–helix protein, is essential for the initiation of *Sxl* activity[1,7,8].

In vitro studies of complexes between DA and bHLH proteins encoded by the *achaete-scute complex* (*AS-C*), of which *sis-b* is a member, suggest that they can bind specific DNA sequences as heterodimers and activate transcription[9–11]. DA protein may also associate with non-HLH proteins, such as the bZIP protein product of *sis-a*, to promote transcription[5]. The amount of maternally provided *da* gene product is expected to be the same in both male and female embryos, but a two-fold difference is expected in the product levels of the zygotically active numerator genes *sis-a*, *sis-b* and *runt*. It appears that heterodimers between DA and SIS proteins activate transcription of *Sxl* and the probability of activation depends on limiting concentrations of SIS proteins (i e. products of *sis-a*, *sis-b* and similar numerators) which are expected to be expressed at levels that are two-fold higher in XX (female) embryos than in XY (male) embryos. SIS levels in males are insufficient to activate *Sxl*.

X-linked numerator elements are 'measured' in reference to the autosomal 'denominator' component of the X : A ratio. Denominator elements, by negatively regulating *Sxl*, would be expected to appear as masculinizing genes that can antagonize the action of the feminizing numerator elements. A candidate denominator gene, *deadpan* (*dpn*), has recently been identified[12,13]. *dpn* codes for a basic helix-loop-helix protein with a HLH domain which closely resembles that of the *hairy* (*h*) protein. *dpn* mutations show male-specific lethality and dose-dependent interactions with *sis-b* and suppress female lethality caused by the numerator gene *sis-a*. Embryos mutant for *dpn* show ectopic *Sxl* expression. Another gene, *extra macrochaetae* (*emc*), which codes for a helix–loop–helix protein, is needed maternally for proper communication of the X : A ratio signal. *emc* acts as a negative regulator of *Sxl*. Males with reduced maternal emc^+ activity and imbalance of $sis\text{-}b^+$ to dpn^+ dosage, i.e. with two copies of $sis\text{-}b^+$ and one copy of dpn^+, show reduced viability[12].

Sex-specific activation of *Sxl* is evident by the blastoderm stage and it seems to occur in an all-or-none fashion. The early transcripts are readily detected in female embryos but not in male embryos[8]. An important feature of the X : A signal in wild-type flies is that the two-fold difference in the dosage of numerator genes elicits a binary, 'male' or 'female', decision. In other words, this mechanism converts the two-fold difference into an all-or-none response. This could come about, for instance, by one of the following mechanisms: (i) a concentration-dependent, co-operative association among HLH proteins encoded by *da*, *sis-b* and similar genes; (ii) the early-acting promoter of *Sxl* may contain multiple binding sites for its activation and thereby facilitate amplification of the X : A signal or (iii) denominator elements of the X : A signal may modulate the activity of the relevant transcription factors acting on the promoter of *Sxl*. *sis-b* and *da*, as well as the negative regulators *dpn* and *emc*, code for HLH proteins that can form homo- or heterodimers with different affinities to specific DNA sequences. Negative regulation may occur by binding of DPN and EMC to DNA directly and blocking transcription or by forming HLH heterodimers with SIS-B that are unable to bind to DNA. For example, if the products of the denominator genes (*emc* and *dpn*) bind and mop up 75% of the relevant products of a single X chromosome, then effective numerator activity could differ five-fold between the sexes (25 in males and 25 + 100 in females).

Recent work has shown that these sex-determining genes, all but one of which encode helix–loop–helix proteins, are also involved in neuronal development. Investigations of the role of these genes in these two pathways have converged to provide an interesting example of multifunctionality of regulatory proteins during development. The HLH proteins encoded by this set of genes seem to interact with one another and with DNA to generate a dose-sensitive switch which determines sexual differentiation by acting on *Sxl* and neuronal cell fates by acting on unknown target genes.

During neuronal development, cells acquire the capability to choose between pathways leading to neurogenesis or epidermogenesis. The gene *da*, in addition to its role in sex determination, plays an essential role in the development of the nervous system. In the latter, as in sex determination, *da* interacts with the genes of the *achaete-scute complex* (*AS-C*). Four bHLH factors of *AS-C* regulate the capacity of ectodermal cells to become neuroblasts in the central and peripheral sensory nervous systems. In the absence of the *achaete-scute* complex of genes, or *da*, some or all of the sensory neuronal precursors fail to form[7,14–18]. *runt* is essential for formation of a subset of neuroblasts, ganglion mother cells and neurons[19]. *emc* and *dpn* also function in both sex determination

and neural development. In the nervous system, *emc* functions as a negative regulator of adult sensory organ development. Loss of function of these genes causes most or all cells in the ectoderm to develop as neuronal precursors, resulting in hypertrophy of the nervous system at the expense of epidermal development. *emc* participates in sensory organ development by antagonizing the neurogenic activity of genes in the *AS-C*. Extra doses of *AS-C* linearly enhance the *h* and *emc* phenotypes, and extra doses of h^+ and emc^+ suppress the excess sensory organs induced by overexpression of *ac* and *sc*. It appears that *h* and *emc* products repress *ac* and *sc* leading to local depletion of these products. This causes spatially restricted activation of *ac* and *sc* genes and the development of sensory organs[20-22]. *dpn* is expressed transiently in most or all neuroblasts[23]. The pattern of expression of *da*, *sis-b*, *runt* and *dpn* is spatially restricted during neurogenesis, but appears to be temporally restricted and spatially uniform for activation of *Sxl*.

HLH transcription factors are also candidate regulators of development in the mammalian nervous system. Homologues of the *Drosophila AS-C* genes have been isolated in several vertebrate species including the rat[24], and mouse[25]. Two such genes, Mammalian *achaete-scute* homologues 1 and 2 (*Mash-1* and *Mash-2*), have been studied in some detail. *Mash-1* expression is restricted to the developing central and peripheral nervous systems, whereas *Mash-2* transcripts are formed exclusively in trophoblast cells. *Mash-1* expression is initially spatially restricted to specific domains of embryonic neuroepithelium. Later, *Mash-1* is more broadly expressed within the ventricular cells. In postnatal animals, expression of *Mash-1* is detected only in regions undergoing neurogenesis, such as the cerebellum and hippocampus. The expression of *Mash-1* generally precedes the appearance of markers of neuronal differentiation such as tyrosine hydroxylase and neurofilaments and, like the expression of *achaete-scute*, appears to be extinguished shortly prior to terminal neuronal differentiation. In mice homozygous for *Mash-1* the olfactory epithelium and the sympathetic, parasympathetic and enteric ganglia are severely affected[24-27].

The occurrence of similar HLH regulatory proteins in organisms as diverse as flies and mammals and their involvement in developmental pathways as different as sex determination and neuronal development suggests both conservation and multifunctionality of these proteins. One might expect to see genetic redundancy[28] among those genes that code for proteins which dimerize with a common target molecule to perform a common function. Partial substitution of the function of one gene by another is in fact observed among *sis-a*, *sis-b* and *runt*[4], the Drosophila sex determination genes which act as numerators for X : A ratio measurement (reviewed in ref. 29).

1. Cline, T. W., Genetics, 1988, **119**, 829–862.
2. Torres, M and Sanchez, L., *EMBO J.*, 1989, **8**, 3079–3086.
3. Torres, M and Sanchez, L., *Genet. Res.*, 1992, **59**, 189–198.
4. Duffy, J B. and Gergen, J. P., *Genes and Development*, 1991, **5**, 2176–2187.
5. Erickson, J. W. and Cline, T. W., *Genes and Development*, 1993, **7**, 1688–1702
6. Villares, R. and Cabrera, C. V., *Cell*, 1987, **50**, 415–424.
7. Caudy, M. *et al.*, *Cell*, 1988, **55**, 1061–1067.
8. Keyes, L. N., *Cell*, 1992, **68**, 933–943.
9. Murre, C. *et al.*, *Cell*, 1989a, **56**, 777–783.
10. Murre, C. *et al.*, *Cell*, 1989b, **58**, 537–544
11. Cabrera, C. V. and Alonso, M. C., *EMBO J.*, 1991, **10**, 2965–2973.
12. Younger-Shepherd, S. *et al.*, *Cell*, 1992, **70**, 911–922.
13. Barbash, D. and Cline, T. W., 33rd *Drosophila Research Conference, Philadelphia*, March 1992 (abstract 18 3).
14. Dambly-Chaudiere, C. and Ghysen, A., *Genes and Development*, 1987, **1**, 297–306.
15. Dambly-Chaudiere, C. *et al*, *Wilhelm Roux' Arch Dev. Biol*, 1988, **197**, 419–423.
16. Bodmer, R. *et al.*, *Neuron*, 1989, **3**, 21–32.
17. Brand, M. and Campos-Ortega, J. A., *Wilhelm Roux' Arch. Dev. Biol.*, 1989, **197**, 457–470.
18. Jan, Y. N. and Jan, L. Y., *Trends Neurosci.*, 1990, **13**, 494–498.
19. Duffy, *et al*, *Development*, 1991, **113**, 1223–1230.
20. Ellis, H. M. *et al.*, *Cell*, 1990, **61**, 27–38.
21. Garrell, J. and Modolell, J., *Cell*, 1990, **61**, 39–48.
22. Van Doren, M. *et al*, *Development*, 1991, **113**, 245–255.
23. Bier, E. *et al.*, *Development*, 1989, **3**, 1273–1287.
24. Johnson, J. E *et al.*, *Nature*, 1990, **346**, 858–861
25. Guillemot, F. and Joyner, A. L., *Mech. Dev.*, 1993, **42**, 171–185.
26. Lo, L.-C. *et al.*, *Genes Dev.*, 1991, **5**, 1524–1537.
27. Guillemot, F. *et al*, *Cell*, 1993, **75**, 463–476.
28. Thomas, J. H, *Trends Genet.*, 1993, **9**, 395–398
29. Anand, A, *A search for additional genes affecting sex determination in Drosophila melanogaster*, Ph D. thesis, Indian Institute of Science, Bangaloie, 1994

ACKNOWLEDGEMENTS. Research in HSC's laboratory has been funded by the Jawaharlal Nehru Centre for Advanced Scientific Research and the Department of Biotechnology, Government of India

The authors are in the Microbiology and Cell Biology Department, Indian Institute of Science, Bangalore 560 012, India

Challenges of user-driven R&D: BARC model*

E. C. Subbarao

It is an honour and privilege to deliver the first P. R. Roy Memorial Lecture. Pradip Ranjan Roy did pioneering work in plutonium technology, beryllium technology and nuclear fuels—fields in which there was hardly any published work to serve as a guide for a newcomer. Starting from scratch and learning everything the hard way, Roy became an international authority in these fields and made India self-reliant in established as well as novel nuclear fuels. He accomplished these in a short span of 23 years with self-effacing modesty. His cheerful and helpful personality enabled him to build a dedicated team to carry on his work.

Roy personifies the philosophy and achievements of Bhabha Atomic Research Centre (BARC), Trombay, Bombay, which is an outstanding example of user-driven research and development in the Indian

*Based in part on the first P. R. Roy Memorial Lecture delivered under the auspices of the Bombay Metropolitan Region Chapter of the Indian Ceramic Society on 24 April 1992, at the Bhabha Atomic Research Centre, Trombay.