Germ line imprinting produces parent-specific differences in the behavior of chromosomes or expression of genes. Epigenetic marks, placed on chromosomes in the parental germ line, govern classical imprinted effects such as chromosomal inactivation, chromosome elimination and mono-allelic expression. Germ line imprinting occurs in insects, plants and mammals. Several Drosophila systems display imprinted effects. In spite of this, many aspects of imprinting in flies, including the normal function of this process, remain mysterious. Transgenerational inheritance of epigenetic marks is a powerful force in genome regulation. Elucidation of the mechanism of imprint establishment and maintenance in a model organism, such as Drosophila, is thus of great interest. In this review we summarize the primary systems that have been used to study imprinting in flies and speculate on the origin and biological function of imprinting in Drosophila.

## Getting in the Last Word

Germ line imprinting is often viewed as the parting gift of a meddling parent. Unable to cede control of genetic material, conditions are placed on its use. These instructions come in the form of epigenetic marks that are deposited on chromosomes in the germ line. Allele-specific regulation of individual genes, or differences in the expression or transmission of entire chromosomes, is the result. Because the sex of the parent determines the presence of these marks, imprinting creates functional differences between the maternally and paternally derived copies of the genome. Imprinting was first described in insects, but has subsequently been observed in a wide range of plants and animals. Imprinted marks in mammals, plants and many insects are necessary for developmentally important processes. While germ line imprinting occurs in flies, a clear understanding of the biological significance of imprinting in Drosophila is still lacking.

Imprinted effects in a wide range of organisms include the heterochromatinization or elimination of chromosomes, transcriptional silencing of a single allele and epigenetic memory. The importance of parental imprints for mammalian embryonic development is illustrated by lethality, and aberrant growth patterns, of diploid androgenetic or gynogenetic zygotes created by pronuclear transfer. In mammals imprints regulate gene expression. Transcriptional silencing of one allele, resulting in mono-allelic expression, is characteristic of these marks. About 100 mammalian genes are imprinted, and these are clustered around Imprint Control Regions (ICRs) that coordinate the imprinted status of nearby genes. Many of these imprints influence genes that regulate embryo and placenta size or developmental processes. For example, methylation at the promoter silences the paternal allele of Igf2r, a scavenger receptor for Igf2 (Insulin like growth factor 2). Reduction in Igf2r increases the concentration of circulating Igf2, thus promoting growth. Imprinting of Igf2r follows a pattern in which paternal imprints tend to increase embryo size but maternal ones limit growth. This has lead to the “parental conflict” hypothesis, which posits that imprinted marks are the means by which parents fight over allocation of resources for their offspring. An extreme example of clustered imprinted genes is the mammalian X chromosome. The paternal X chromosome is silenced in marsupials and in extraembryonic tissues of rodents. Silencing of the paternal X chromosome expeditiously achieves equalization of X-linked gene dosage, known as dosage compensation, between males and females. In the inner cell mass of placental mammals the imprint is erased, enabling random X inactivation. While the imprinted mark regulates an entire chromosome, the imprint itself need only control the X inactivation center (Xic), a locus on the X chromosome that directs inactivation. Through regulation of growth, development and X chromosome silencing, mammalian imprints direct multiple essential processes during early embryogenesis. Compelling arguments for the adaptive value of imprinting have consequently focused on its central role in mammalian development.

While the ramifications of imprinting are best understood in mammals, imprinting itself was first described in Sciarid flies. In contrast to the gene expression effects observed in mammals and plants, imprinting in insects often controls the behavior of entire chromosomes. For example, in Sciara, imprinting directs heterochromatinization of the paternal X chromosome and its elimination in the germ line and soma. This elimination is attributable to a single controlling element near the centromere,
now referred to as the imprinting control region (ICR). The ICR is a common feature of many imprinted loci, including Xic in mammals. Other examples of insect imprinting involve silencing of the entire paternal genome of male mealybugs by heterochromatinization. In these scale insects, females are diploid and males are pseudohaploid, meaning that somatic cells are functionally haploid as a consequence of silencing. Imprinting thus plays essential roles in sex determination and meiosis in insects. Several examples of imprinting have been documented in Drosophila, but the manifestations of imprinting in flies are markedly different from those in the organisms described above.

**Several Methods of Detection Reveal Imprinting in Drosophila**

As in other insects, Drosophila imprints are detected through their effects on heterochromatin. Imprints in flies can affect entire chromosomes, but they are usually detected by silencing of a euchromatic reporter that has been moved near heterochromatin by transposition or chromosome rearrangement. This silencing, termed position effect variegation (PEV), produces patches of tissue in which spreading heterochromatin has silenced the reporter. Structurally normal chromosomes presumably have insulators that prevent the spread of heterochromatin into euchromatic regions. While most instances of PEV are not affected by imprinting, in a few the parent of origin dramatically influences the amount of silencing. Examples include the expression of variegating genes on the rearranged Dp(1;f)LJ9 and Dp(1;f)LJ9 (mini-X) chromosomes. Dp(1;f)LJ9 has been used extensively to explore the mechanism of imprinting in flies. It was created by complex rearrangements that delete most of the X euchromatin and move a group of euchromatic genes, including garnet (g+), close to proximal heterochromatin (chromosome model, Fig. 1A). As Dp(1;f)LJ9 is a free duplication of part of the X chromosome, it can be transmitted from either parent. Maternal transmission results in uniform expression of g+, producing solid red eyes. Transmission from the father produces orange patches in which g+ has been silenced. PEV is thus observed only upon paternal transmission.

Transgene insertions on the heterochromatic Y chromosome are also subject to PEV. Unlike the situation in mammals, the fly Y chromosome does not determine sex, and the known functions of Y-linked genes are limited to spermatogenesis. The Y chromosome of Drosophila can thus be transmitted through females. Y-linked insertions typically display greater expression when transmitted through a female. This is illustrated by the more uniform expression of w+ and y+ markers on maternally transmitted Y-linked insertions (Fig. 1B). Other parent-of-origin effects mediated by epigenetic marks deposited in the parental germ lines have also been noted. These include loss of paternal chromosomes in pal mutant progeny and defects caused by the Uab1 inversion of the bithorax complex. The chromatin structure of the bithorax complex is organized into repressed and active chromatin domains. Imprinting of the Uab1 inversion may reflect changes in the chromatin organization at this locus, perhaps analogous to the effect of imprinting on PEV.
An interesting imprinting-like effect on the Y chromosome is observed in mutants of E(var)3-93D, also known as mod(mdg4). mod(mdg4) was one of the first enhancers of PEV identified and is required to maintain an open chromatin conformation.23,24 The variegation of eye color in w<sup>ex6</sup> flies, in which w<sup>+</sup> has been moved near heterochromatin by inversion, is enhanced in mod(mdg4) mutants. A Y chromosome transmitted through a mod(mdg4) male also enhances variegation of the w<sup>ex6</sup> allele.26 However, the effect of the Y chromosome is maintained for many generations, even when transmitted through wild type flies. Because this appears to be a permanent change in the Y chromosome, it does not meet the definition of germ line imprints, which are reset every generation as they pass through the germ line.

Surprisingly, imprinting of the Y chromosome can also influence X chromosome dosage compensation.35 A two-fold increase in expression from the male X chromosome is required to equalize X-linked gene expression between males and females. Two non-coding roX RNAs (roX1 and roX2) are components of a ribonucleoprotein complex that achieves this by binding to the X chromosome and modifying chromatin.36 The roX RNAs are required for recognition of X chromatin.27-29 Simultaneous mutation of roX1 and roX2 leads to reduced X-linked gene expression and low male viability.30 Although the Y chromosome has no effect on dosage compensation in otherwise wild type flies, a maternally transmitted Y chromosome dramatically suppresses the lethality of roX1 roX2 males (Fig. 1C).25 The mechanism by which this occurs remains under investigation, but expression of X-linked genes is modestly increased in roX1 roX2 males with a maternal Y chromosome. Male rescue is presumably due to this increase in expression. The Y chromosome imprint is reset each generation, and thus is a true germ line imprint (Menon DU, unpublished).

How do Flies Imprint?

The process of establishing and maintaining imprints in flies remains mysterious. In contrast, well-studied mammalian examples reveal that imprint establishment and maintenance each rely on DNA methylation. Mammalian germ line imprints consist of specific methylation patterns established in the gametes by DNMT3A (DNA methyltransferase) and DNMT3L, a non-catalytic co-factor.31-33 After fertilization, these allele-specific patterns are propagated in the soma by the maintenance methyltransferase DNMT1.34,35 Establishment and maintenance of fly imprints are separately regulated. While the establishment of imprints remains mysterious, factors that influence heterochromatin formation have been shown to affect the maintenance of the imprint. Loss of heterochromatin proteins like HP1, and Su(var)3-9 (H3K9 methyl transferase) suppress expression of the paternal imprint, while mutation of trithorax (trx) and Brahma (brm), proteins that activate gene expression, suppresses the maternal imprint.36 Cytological studies have shown that when paternally transmitted, Dp(1;f)LJ9 undergoes less endoreplication in the salivary gland and has more uniformly compact chromatin than when it is maternally transmitted.43 Taken together, these studies reveal that fly imprints are capable of exerting long-range effects on gene expression, chromosomal replication and chromatin structure that are maintained throughout the life of the organism. Surprisingly, given the fact that imprints appear to be placed in heterochromatic regions, factors known to influence heterochromatin do not appear to affect establishment of imprints.32,34

Although the nature of the imprint itself remains unknown, it is possible that imprints are placed by transient signals that influence heterochromatin. Maintenance of heterochromatin could then perpetuate the imprinted state throughout the life of the animal. While heterochromatic imprinting is characteristic of flies, a recent study in mice revealed that the establishment of pericentric heterochromatin depends on the parent of origin.45 Sperm DNA is compacted with protamines. Following fertilization, protamines are removed and the male pronucleus is assembled with maternal proteins. In the zygote, heterochromatin of the maternal genome is enriched for H3K9me3, a mark made by Su(var)39, and HP1, which binds H3K9me3. Paternal heterochromatin lacks this signature, and instead is enriched for H3K27me3, a mark deposited by the Polycomb-repressive complex 2 (PRC2). This mark recruits the PRC1 complex, necessary for inhibition of transcriptional activation. Formation of paternal heterochromatin requires the maternal PRC1 complex, and, in its absence, transcription of paternal satellite repeats is derepressed. Asymmetry in heterochromatin
establishment has the potential to be a general imprinting mechanism, employed by any organism that restructures a male pronucleus. Imprinting of heterochromatic regions thus could be more widespread, and evolutionarily older, than previously thought.

Given the importance of heterochromatin for fly imprinting, understanding heterochromatin formation is essential. RNAi was first shown to regulate heterochromatin formation in the fission yeast, Schizosaccharomyces pombe. Mutations affecting RNAi also disrupt heterochromatin formation in Drosophila. Multiple RNAi pathways have been shown to regulate heterochromatin formation in the soma and germ line. Transcripts from repetitive regions are processed into siRNA, which in turn direct silencing chromatin marks to these regions. The role of RNAi in initiation of heterochromatin formation makes it a likely candidate for involvement in imprinting.

Insulators, such as CTCF, that establish higher order chromatin structure by regulating looping and position within the nucleus, are also candidates for a role in imprinting. Insulators act as barriers, preventing heterochromatin spreading and blocking promoter-enhancer interactions in mammals and Drosophila. This contributes to CTCF function in imprinting of Igf2/H19, and in organization of chromatin domains in the fly Bithorax complex. It is possible that proteins with insulator function in flies will also affect imprinting. Drosophila has several insulator proteins, including CTCF and SU(Hw), which binds to gypsy elements and influences looping and nuclear localization. Despite the fact that these two insulators bind distinct sequences, CTCF and SU(HW) co-localize to insulator bodies, complex nuclear structures that anchor loops to organize multiple, large chromatin domains. The ability of insulators to control large chromatin domains, and the central role of CTCF in mammalian imprinting, makes these proteins attractive candidates for a role in establishment or interpretation of imprints in Drosophila.

Why do Flies Imprint?

The presence of germ line imprinting in Drosophila is intriguing, but the biological function of these imprints remains mysterious. Studies of imprinting in several other organisms have lead to an understanding of the role of imprinting in these species. Because failure of imprinting in mammals causes a wide range of developmental defects, we now understand the importance of monoallelic expression of imprinted genes for early mammalian development. Imprinting in Sciara and scale insects guides the behavior of entire chromosomes, playing a vital role in meiosis and sexual differentiation. In Drosophila, imprints are detected by alteration in expression of genes on rearranged chromosomes, but there is little to suggest that expression of any gene in karyotypically normally flies is governed by imprinting. Indeed, genome-wide expression analysis of progeny from reciprocal crosses of inbred strains suggests that gene expression differences that appear to depend on the parent of origin do not arise from monoallelic expression, but are more likely due to maternal or paternal effects.

A compelling argument for the origins of imprinting has been made by de La Casa-Esperon and Sapienza. These authors suggest that imprinting serves to identify homologous chromosomes and sister chromatids, a distinction important during DNA repair and meiotic recombination. Unscheduled double stranded breaks may be fixed by gap repair, using a template from another chromosome. Holliday structures join the damaged and template chromosome. When a homologue is the template, resolution of the Holliday structure can result in mitotic recombination. This has potentially serious effects as it can uncover deleterious recessive mutations. This danger is not present when the template for repair is a sister chromatid. Indeed, cells favor the sister chromatid when undergoing this type of repair. In contrast, recombination between homologues is usually essential for chromosome segregation during meiosis. Cells thus have compelling reasons to distinguish homologues from sister chromatids. Marks placed on chromosomes in the parental germ line, and maintained throughout the life of the organism, may enable cells to make this distinction. The function of imprints in various types of gene expression might have arisen by taking advantage of existing marks that distinguish homologous chromosomes.

There is also support for a different origin of germ line marks. Imprinting in flies is usually studied in organisms with rearranged chromosomes, raising the possibility that these rearrangements are required for deposition of some imprinted marks. One of the consequences of chromosomal rearrangement is the disruption of normal chromosome pairing. Interestingly, chromatin that is unpaired during meiosis is sometimes modified. This occurs in Neurospora, where unpaired DNA creates a signal that silences identical sequences. In C. elegans, chromatin that is unpaired in the germ line acquires silencing marks that are retained through early zygotic development. Silencing of unpaired chromatin in Neurospora and the deposition of silencing marks in C. elegans may have arisen to inactivate mobile elements. The disruption of pairing by rearrangements might similarly be necessary for deposition of germ line imprints. This idea is supported by investigations of the variegating In(1)ac8 chromosome. Greater variegation of y and ac was observed in the offspring of mothers that were heterozygous for the rearrangement, rather than homozygous. If marks deposited on unpaired chromosomes establish Drosophila imprints, the Y chromosome is an obvious target. In support of this idea, the Y chromosome is imprinted even when it is not rearranged. As the Y chromosome is entirely heterochromatic, it provides an excellent target for epigenetic marks that require heterochromatin.

These speculations about the origin of imprinted effects in flies raise the possibility that the differences in gene expression that characterize imprinted fly chromosomes may have little relation to the biological function of imprinting in this organism. Even though the origin and molecular basis of imprinted effects in Drosophila are not yet understood, it is clear that mechanisms for imprinting exist in flies, and imprinted marks regulate chromatin throughout the life of the organism. Drosophila shares epigenetic processes, such as heterochromatin formation, RNAi-directed chromatin regulation, insulation and possibly DNA methylation, with other organisms. Imprinting in flies is a fascinating and potentially powerful system in which to study transgenerational inheritance and propagation of these marks.