The Additional sex combs gene of Drosophila is required for activation and repression of homeotic loci, and interacts specifically with Polycomb and super sex combs

Abstract The protein products of Polycomb group (PcG) and trithorax group (trxG) genes are required for the maintenance of the transcriptionally repressed and active states, respectively, of the homeotic genes. Mutations in PcG genes produce gain-of-function (posterior) homeotic transformations, while mutations in trxG genes produce loss-of-function (anterior) homeotic transformations. Double mutant combinations between a PcG gene and a trxG gene suppress the homeotic transformations seen with either mutation alone, suggesting that PcG and trxG genes act antagonistically. The PcG gene Additional sex combs (Asx) is interesting because one mutant allele, Asx\(^{P1} \), causes both anterior and posterior homeotic transformations. Asx\(^{P1} \) and other Asx mutations were crossed to mutations in the PcG gene Polycomb (Pc) and the trxG gene trithorax (trx). Asx alleles enhance both PcG and trxG homeotic transformations, showing that Asx is required for both the activation and the repression of homeotic loci. Asx also shows strong allele-specific interactions with the PcG genes Pc and super sex combs (sxc). Together, these data indicate that there are functional interactions between Asx, Pc and sxc in vivo. ASX may interact with a PcG complex containing PC and SXC and mediate activation versus repression at target loci, perhaps by interacting directly with the TRX protein.

Key words Polycomb group · Trithorax group · Homeotic mutations · Drosophila melanogaster

Introduction

Proteins encoded by the Polycomb group (PcG) genes maintain the spatially restricted expression patterns of the homeotic genes by repressing the homeotic genes outside their normal expression domains (Simon 1995; Pirrotta 1997). In PcG mutants, homeotic gene expression patterns are initially normal but the homeotic genes become ectopically expressed as development proceeds, indicating that the PcG genes are required for maintenance but not for the initiation of homeotic gene repression (Struhl and Akam 1985; Jones and Gelbart 1990). In most cases, homozygous PcG mutations are lethal; the homozygotes die at the end of embryogenesis. However, the ectopic expression of homeotic genes in heterozygous PcG mutants produces gain-of-function homeotic mutant phenotypes in adult flies (Simon 1995). Most homeotic genes are expressed in domains with sharp anterior boundaries so that when expression boundaries break down, homeotic gene expression expands in an anterior direction and transforms anterior structures into more posterior ones. An exception to this is the product of the homeotic gene Sex combs reduced (Scr), expression of which expands in both an anterior and a posterior direction (Glicksman and Brower 1988), producing extra sex combs on the second and third legs of male flies. This dominant extra sex combs phenotype has been used to identify many members of the PcG, as well as modifiers of PcG activity. Double mutant combinations between PcG genes enhance the extent to which this phenotype is seen (Jürgens 1985). The number of legs that display extra sex combs is a measure of the strength of the mutant interaction and has been used in the past in an attempt to divide the PcG into specific functional subsets (Cheng et al. 1994; Campbell et al. 1995).

Proteins encoded by the trithorax group (trxG) genes are required to maintain the activation of homeotic genes within their normal expression domains. Mutations in trxG genes reduce the levels of homeotic gene
expression and produce loss-of-function homeotic transformations. Because there are many ways to reduce levels of gene expression, the trxG is likely to be more heterogeneous than the PcG (Kennison 1993, 1995). Mutations in members of the trithorax group (trxG) of genes reduce homeotic gene expression within their normal domains and generally cause posterior structures to be transformed into more anterior ones (Kennison and Tamkun 1988; Breen and Harte 1993; Kennison 1993). An exception to this is the transformation of the first and second legs towards the third leg, presumably due to a reduction in Scr expression (Ingham and Whittle 1980). This is most easily monitored as suppression of the formation of sex combs on the first legs of male flies. As with members of the PcG, double mutant combinations between two trxG genes exhibit enhanced penetrance of trxG homeotic transformations, suggesting that some trxG gene products may function together in maintaining the activation of the homeotic loci (Kennison and Russell 1987; Kennison and Tamkun 1988; Shearn 1989).

Many trxG mutations were initially isolated as suppressors of the PcG extra sex combs phenotype (Kennison and Tamkun 1988). Generally, double mutant combinations between PcG and trxG genes lead to the suppression of the homeotic transformations seen with mutations in either group alone (Ingham 1983; Capdevila et al. 1986; Kennison and Tamkun 1988), suggesting that PcG and trxG proteins either have opposite and independent functions (Ingham 1983) or function antagonistically to one another, possibly via stoichiometric competition for repression or activation at specific target loci (Jones and Gelbart 1993).

In Drosophila, there are 15 PcG loci, of which 13 have been cloned and sequenced (Simon 1995; Pirrotta 1997). The PcG gene Additional sex combs (Asx) was originally identified in a screen for embryonic lethal mutations that caused pattern defects in the larval cuticle (Nüsslein-Volhard et al. 1984; Jürgens 1985) and independently as a mutation that enhances the dominant sex combs phenotype of Polycomb (Pc) mutants (Dura et al. 1985). Homozygous Asx mutants die at the end of embryogenesis, and exhibit severe head defects and mild posterior transformations in the cuticle of the abdomen (Jürgens 1985; Breen and Duncan 1986; Sinclair et al. 1992). Based on the modest posterior transformations, Asx was categorized as a weak member of the PcG. In agreement with this observation, homeotic derepression phenotypes in the central nervous system are less severe in Asx mutant embryos than those seen with mutations in most other PcG genes (McKeon and Brock 1991; Simon et al. 1992; Sinclair et al. 1998), even when the maternal contribution of Asx is removed (Soto et al. 1995). Asx encodes a chromatin protein with a cysteine cluster that is conserved in mammals (Sinclair et al. 1998).

The extra sex combs phenotype is rarely seen in heterozygous Asx mutant adults (Sinclair et al. 1992), but Asx can strongly enhance the extra sex combs phenotype caused by mutations in other PcG genes. In particular, the Asx allele Df(2R)trix strongly interacts with mutations in Pc to produce flies with multiple sex combs (Campbell et al. 1995). One allele of Asx, AsxP1, (a P element insertion in the 5' untranslated region of Asx), is unique because it causes both anterior (trxG) and posterior (PcG) transformations in adult flies (Sinclair et al. 1992). The AsxP1 allele is homozygous viable but it is semi-lethal with strong Asx alleles. The paradoxical dual phenotype of the AsxP1 allele indicates that Asx may have a role in both repression and activation.

To determine if Asx could function as both a member of the PcG and the trxG, AsxP1 homozygotes were tested for interactions with both Pc and alleles of the trxG gene trithorax (trx). Flies homozygous for AsxP1 and heterozygous for Pced showed strong enhancement of PcG phenotypes, whereas flies homozygous for AsxP1 and heterozygous for trx alleles exhibited strong enhancement of trxG phenotypes. To determine if this dual phenotype was specific to the AsxP1 allele or was a reflection of a general requirement for Asx activity in both repression and activation, lethal Asx alleles were crossed to Pc and trx alleles and found to enhance both PcG and trxG homeotic transformations. Df(2R)trix, a true null allele of Asx, showed an especially strong interaction with Pc not seen with other PcG mutations. Alleles of Asx also show a strong genetic interaction with a specific allele of the PcG gene sxc. This indicates that there may be specific in vivo functional interactions between Asx, Pc and sxc. This suggests a model in which the ASX protein interacts with a PcG complex containing the PC and SXC proteins and mediates activation versus repression at target loci, perhaps by interacting directly with the TRX protein.

Materials and methods

The fly strains used in this study are described in Lindsley and Zimm (1992) and on Flybase (http://flybase.bio.indiana.edu). AsxP, AsxP1, AsxP1 are homozygous lethal alleles described by Jürgens (1985) or Sinclair et al. (1992). Because these Asx alleles have stronger phenotypes than a deletion of Asx, they are probably gain-of-function alleles. The AsxP1 allele is heterozygous viable and is described in Sinclair et al. (1992). The Asx deficiency Df(2R)trix is described in Breen and Duncan (1986). Df(3R)red is a large deletion the removes the trx locus. The AsxP1 allele has a deletion in the coding region of trx and is probably a null, and trxP1 is a point mutation in the SET domain, a protein domain that is conserved in other regulatory proteins (Stassen et al. 1995). Pced is a strong Pc allele originally considered to be a null. All sxc alleles are described in Ingham (1984). The AsxP1 chromosome carries the recessive eye-color mutations cn and bw, so homozygous AsxP1 flies are identifiable by their white eyes. An AsxP1cn bw/AsxP1 cn bw; Ly/TM3 stock was constructed for these studies.

All crosses were performed at 25°C. Flies were raised on standard cornmeal-sucrose medium containing Tegosept as a mold inhibitor. Some 15–20 females were crossed to 15–20 males of the appropriate genotypes. Crosses were transferred once after allowing the females to lay eggs for 4 days. Parents were discarded after 4 more days and the F1 were allowed to enclose. F1 flies were then scored at 2-day intervals over a 10-day period.