Somatic and germline clone analysis in mutants of the *S*-adenosylmethionine synthetase encoding gene in *Drosophila melanogaster*

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Abstract We have analysed the phenotypic consequences of homozygous mutant clones in the *S*-adenosylmethionine synthetase encoding gene in *Drosophila melanogaster*. The results suggest that SamS function is required for cell proliferation/growth in embryonic/early larval cells and during development of imaginal disc cells. Homozygous *SamS* germline clones can, however, develop and give rise to viable heterozygous offspring. This offspring expresses a *Minute*-like phenotype. We suggest that this phenotype is caused by an obstruction of the polyamine biosynthesis.

Key words: *S*-Adenosylmethionine synthetase; Polyamine synthesis; Protein synthesis; *Drosophila melanogaster*

1. Introduction

S-Adenosylmethionine synthetase (SamS) (EC 2.5.1.6) is the enzyme that catalyses the biosynthesis of *S*-adenosylmethionine (AdoMet) [1]. AdoMet plays a central role in innumerable biological events as a methyl group donor [2]. After decarboxylation, AdoMet also serves as the sole propylamino group donor in the biosynthesis of polyamines. Since AdoMet functions both in methylation reactions and in polyamine synthesis, therapeutic interventions using this molecule and its biosynthesis are attracting attention [2]. For example, claims have been made for using AdoMet itself to treat psychiatric depression [3,4] and inhibition of polyamine biosynthesis has been suggested as a cure for cancer and parasitic infections [5,6]. The myriad of biological effects that involve AdoMet motivates a thorough investigation of its role and function in developmental processes as well.

We have previously [7,8] cloned and characterised the gene encoding *SamS* in *Drosophila melanogaster*. Mutants of *SamS* showed phenotypes that indicate a role for SamS in the regulation of chromatin structure, in particular the observations that *SamS* mutations suppress position-effect variegation (PEV) and enhance the phenotype of *Polycomb* (*Pc*) [8]. Since *Polycomb*-group genes (Pc-G) are believed to be responsible for maintaining the repressed state of homeotic genes [9,10], we were interested to see whether a *SamS* mutation in itself can induce ectopic expression of homeotic genes or if the interaction with *Pc* is a specific enhancer effect.

Homozygous *SamS* mutants are lethal in the embryonic and early larval stages. This lethality restricts the study of possible effects from *SamS* homozygosity in later stages and in the germline. Here, we report the results from analyses of somatic and germline *SamS*/*SamS* clones induced by the FLP site-specific recombination system.

2. Materials and methods

2.1. Drosophila stocks and culture

For a description of mutants used, see [8,11–13]. Stocks were kindly provided by the Bloomington and the Umeå *Drosophila* Stock Centres. All crosses were repeated at least twice. Crosses were made in vials with potato mash-yeast-agar medium at 25°C.

2.2. Immunocytochemistry on whole mount embryo

Embryos from wildtype (Oregon R) and the four *SamS* strains *l(2)M6/CyO P[ftz:lacZ], l(2)R23/CyO P[ftz:lacZ], Su(z)5/CyO P[ftz:lacZ], Df(2L)PM44/CyO P[ftz:lacZ]* were fixed for 20 min in formaldehyde and stained as described [14]. The embryos were double-stained; for the first staining, rabbit antiserum against β-galactosidase (Cappell) at dilution 1:2000 was used. Biotinylated secondary antibody (Jackson) was used at 1:300 dilution and detected by the black HRP reaction (H_2O_2, DAB, Ni^{2+}). Following this, the embryos were stained using antibodies against the homeotic gene products Antp, Ubx and Scr. Mouse monoclonal anti-Antp, anti-Scr and anti-Ubx, which were kindly provided by T. Kaufman, were used at dilutions 1:50, 1:5 and 1:75 respectively. Biotinylated secondary antibody (Jackson) was diluted 1:300 and detection was achieved through the brown HRP reaction (H_2O_2, DAB). Embryos lacking black *ftz-lacZ*-derived staining were classified as homozygous for *SamS*, and analysed.

2.3. Somatic clones

Females carrying somatic clones of *SamS* were generated by use of the FLP-induced recombination technique [13]. Stocks of the genotype *y w; SamS P[ry$^+$ hs-neo FRT]40A/CyO* were constructed. Two different presumed amorphic *SamS* mutant alleles were used: *l(2)M6* and *Su(z)5* (described in [8]). Virgin females from these stocks were crossed with *y w P[ry$^+$ hsFLP]; P[w$^+$] P[ry$^+$ hs-neo FRT]40A/CyO* males. At the age of 24–48 h, the progeny was heat-shocked at 37°C for 80 min. Adult females of the genotype *y w/y w P[ry$^+$ hsFLP]; SamS P[ry$^+$ hs-neo FRT]40A/P[w$^+$] P[ry$^+$ hs-neo FRT]40A* were selected and classified; see Fig. 2A for crossing scheme.

2.4. Germline clones

Females carrying *SamS* germline clones were generated by using the FLP-induced recombination technique in combination with the *P[ovo^{D1}]* dominant female-sterile insertion in the tip of chromosome 2L [12,13]. Virgin females with the genotype *y w/y w; SamS P[ry$^+$ hs-neo FRT]40A/CyO* were crossed with *y w P[ry$^+$ hsFLP]; P[w$^+$ ovo^{D1}] P[w$^+$ ovo^{D1}] P[ry$^+$ hs-neo FRT]40A/CyO* males. The progeny was heat-shocked at 37°C for 80 min, at the age of 24–48 h. Females of the genotype *y w/y w P[ry$^+$ hsFLP]; SamS P[ry$^+$ hs-neo FRT]40A/ P[w$^+$ ovo^{D1}] P[w$^+$ ovo^{D1}] P[ry$^+$ hs-neo FRT]40A* were selected and crossed with *y w*; +/+ males. Any fertility must result from induced germline *SamS*/*SamS* clones. To detect possible leakage of the *ovo^D*-induced sterility, eye colour was monitored in the offspring. The *ovo^D* efficiency was also controlled by using the same strain but without the heat shock. Offspring from females with *SamS*/*SamS* germline clones were analysed for external phenotypes.

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Fig. 1. Expression pattern of the Scr protein. A: A wildtype embryo stage 16–17. B: A homozygous *SamS* embryo, *l(2)M6/l(2)M6*, stage 16–17.

As a control for the F_2 phenotypes we used a heterozygous *SamS* strain which resulted in offspring with the same genotype but with $SamS^+$ maternal contribution. See Fig. 2B for crossing scheme.

3. Results

3.1. Analysis of SamS/SamS embryos

The *SamS* mutant alleles *l(2)M6, l(2)R23, Su(z)5* and *Df(2L)PM44* are recessive lethals and die in late embryonic and in first instar larval stages (results not shown). We have earlier shown that *SamS* mutants enhance the *Pc* mutant [8], which urged us to investigate if *SamS* mutants alone were capable of inducing ectopic expression of the homeotic genes. We therefore stained embryos from *SamS/CyO P[ftz:lacZ]* stocks using antibodies against β-galactosidase (to exclude embryos containing the balancer *CyO* chromosome) and subsequently with one antibody against a homeotic gene product. There was no detectable ectopic expression of Antp, Scr or of Ubx in homozygous embryos from any of the *SamS* alleles tested, not even in late embryonic stages (Fig. 1) (Antp and Ubx not shown). We could not detect any visible embryonic defect that could account for the lethality.

3.2. Somatic SamS/SamS clones

Since the absence of a distinct phenotype in lethal embryos and larvae suggests that the *SamS* mutant in homozygous condition results in a stop in cell proliferation/growth or cell death, we decided to study if this effect was restricted to embryonic/early larval development or if it was a more general effect on cells in other stages as well. In order to induce somatic recombination using the FLP site-specific recombinase we constructed strains with a *SamS P[FRT]* chromosome, (for description see [13]). The crosses are outlined in Fig. 2A. If homozygous *SamS* clones were viable this would result in eyes with twin spot clones, i.e. red clones (w^+/w^+) and white clones (*SamS/SamS*) on a yellow background (w^+/*SamS*). If, on the contrary, *SamS/SamS* causes cell death or interferes with cell proliferation/growth also in the eye antennal imaginal disc, somatic recombination would result in red clones only. As seen in Table 1, the latter turned out to be the case, not a single one of the *SamS* mutant female offspring with somatic eye clones showed any white (*SamS/SamS*) clones.

3.3. Germline SamS/SamS clones

We showed by Northern blot analysis [7] that *SamS* expression is high in ovaries. Maturation of ovaries has been observed to be delayed in *SamS*/+ females (J. Larsson, unpublished results). The restrictions imposed by the embryonic lethality of *SamS* homozygotes on studies of effects in *SamS/SamS* ovaries can be overcome by using the ovo^D dominant female sterility system in combination with FLP/FRT recombination [12,13]. The crossing strategy is outlined in Fig. 2B. Surprisingly, the heat-shocked *y w/w P[hsFLP]; SamS P[FRT]/P[*w^+ ovo^D*]* females were fertile. The possibility that these females represent leakage of the ovo^D-induced sterility was excluded by monitoring the eye colour of the offspring. The *P[*w^+ ovo^D*]* marker also contains w^+ which would result in an orange eye colour of such 'leaky offspring'. No w^+ offspring was found which indicates a complete sterility in ovo^D germline tissue. Omitting the heat shock during the larval stage also resulted in sterility. Therefore, in contrast to the eye antennal disc *SamS/SamS* clones, germline clones can develop and give rise to mature ovaries.

The offspring derived from maternal *SamS/SamS* were analysed for external phenotypes and the results are presented in Table 2. Of the female offspring, 43% showed a phenotype which we called *Minute*-like bristles. The bristles were short and often thinner than normal and occasionally scutellar bristles were absent (Fig. 3A). There was often a left/right asymmetry, i.e. one side of the thorax and the scutellum was more affected than the other (Fig. 3A). The phenotype was not discrete but overlapped with wildtype. We therefore decided to classify the phenotype on the basis of bristle length. Individuals with posterior scutellar bristles that were too short to cross each other were classified as *Minute*-like. 41% of the 43% females classified as belonging to the *Minute*-like class also exhibited fused tergites. We used two *SamS P[FRT]/CyO* strains as control. Both showed a low frequency of flies with *Minute*-like phenotype. In male offspring, 25% showed *Minute*-like bristle phenotype, out of which 23% also had

Table 1
Somatic clones in eyes of F_1 female offspring from the cross outlined in Fig. 2A

SamS allele	% Individuals $w^+/-$; no clones (No. of individuals)	% Individuals w^+/w^+ clones (No. of individuals)	% Individuals w^+/w^+ and $-/-$ clones[a] (No. of individuals)
l(2)M6	33 (32)	67 (66)	0 (0)
Su(z)5	31 (18)	69 (40)	0 (0)
$SamS^{+b}$	19 (70)	44 (158)	37 (134)

[a] $-/-$ represents *SamS/SamS* clones in the mutant crosses.
[b] *y w P[hsFLP]/y w; P[*w^+*] P[FRT]/ P[*y^+*] P[FRT].*

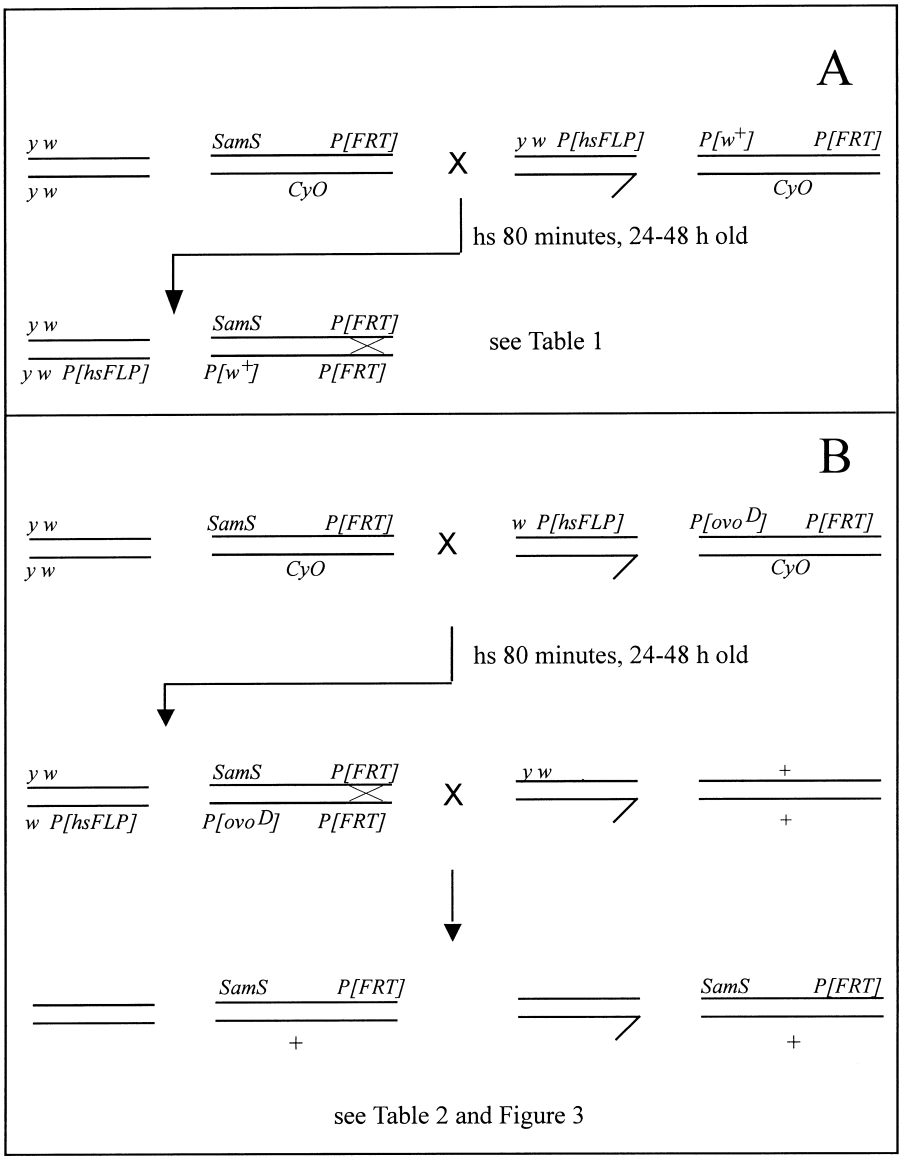

Fig. 2. Crossing schemes for generation of mosaic flies. A: Generation of females with somatic clones. B: Generation of females with germline clones.

fused tergites. In addition to the bristle phenotype 11% of all males also showed rotated genitalia, which protruded from the body (Fig. 3C). In extreme cases, a 180° rotation was observed.

4. Discussion

Polyamines have been shown to be essential for cell growth and differentiation [1,15,16]. Our earlier studies showed that heterozygous *SamS* females have reduced amounts of the polyamines spermine and spermidine in their ovaries [8]. This indicates that *SamS* mutations inhibit polyamine biosynthesis. It can be speculated that the death of *SamS* homozygous embryos is caused by a stop in cell growth and proliferation due to polyamine depletion. However, other effects have to be considered since AdoMet is also a major methyl group

Table 2
Phenotypes of F_2 offspring from the crosses outlined in Fig. 2B

SamS mutant	% Individuals wildtype (No. of individuals)	% Individuals *Minute*-like bristles and normal genitalia (No. of individuals)	% Individuals *Minute*-like bristles and rotated genitalia (No. of individuals)
l(2)M6, female offspring	57 (286)	43 (218)	–
l(2)M6, male offspring	65 (250)	25 (96)	11 (41)
Control 1[a]	94 (90)	6 (6)	0 (0)
Control 2[a]	99 (73)	1 (1)	0 (0)

[a]Two independent *y w; l(2)M6 P[FRT]/CyO* strains.

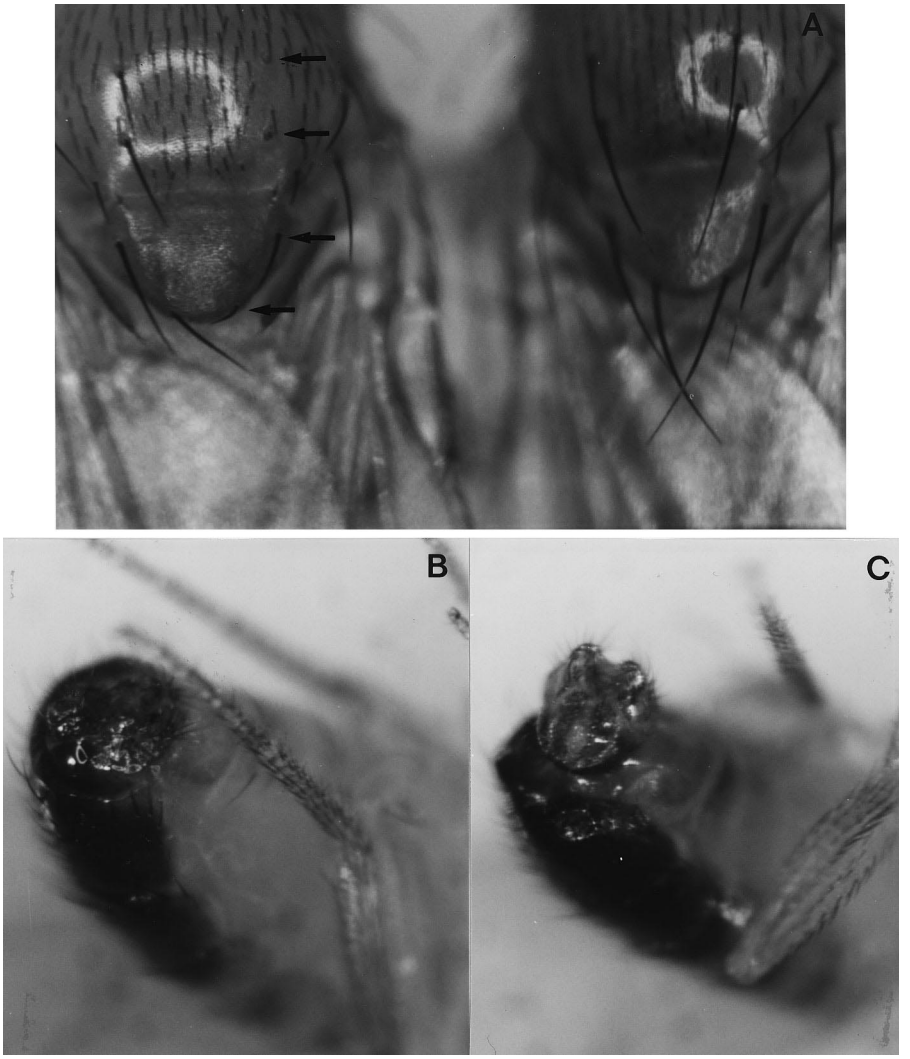

Fig. 3. Phenotypes of *SamS*/+ offspring derived from a *SamS*/*SamS* female germline clone. A: Toraces of *SamS*/+ (left) and wildtype (right) individuals. Note the asymmetry in the mutant both on anterior and posterior scutellars and on dorso-central bristles (arrowheads). B: The genitalia of a wildtype male. C: The rotated protruded genitalia of a *SamS*/+ male.

donor and the biological effects of disturbed methylation are myriad.

We have earlier shown that *SamS* is an enhancer of *Pc* [8]. The present analysis of homozygous *SamS* embryos does not show any homeotic transformation nor any ectopic expression of the homeotic genes *Antp, Scr* or *Ubx*. This indicates that *SamS* should not be included in the Pc-G but rather that it has a function in modifying the effects of *Pc*.

The analysis of homozygous somatic clones in the eyes of adult females indicates that the SamS function is needed for cell proliferation/growth also in later stages of development. In the germline, however, *SamS*/*SamS* clones can develop into functional eggs. The difference between somatic mutant clones and germline clones may be due to the different origin of the ovary cells. The oocyte and the 15 nurse cells originate from the germline. The surrounding follicle cells are somatically derived [17]. It can be speculated that a low level of AdoMet contribution from the follicle cells is sufficient for maturation of the egg. The absence of nurse cell contribution of AdoMet may, however, cause the *Minute* and the rotated genitalia phenotypes.

Offspring from *SamS*/*SamS* clones showed a *Minute*-like phenotype. The *SamS* mutant allele *Su(z)5* is also known as *M(2)21AB* [11,18] but the *Minute* phenotype has been reported to be lost from the stock [8]. However, in stocks where the mutant chromosome has been allowed to recombine, the *Minute*-like bristle phenotype is evident in some flies (i.e. controls in Table 2 and results not shown). This suggests that the *Minute* phenotype is still present but with a low and variable penetrance and expression. This indicates that the *Minute*-like bristle phenotype is not solely attributed to a maternal effect but is a combination of a maternal lack of *SamS* with a zygotic heterozygosity. The observed *Minute*-like phenotype is intriguing when we recall that SamS is needed for biosynthesis of polyamines. Interestingly, we found similar phenotypes when characterising mutants in another gene in the polyamine biosynthesis pathway, namely, *S*-adenosylmethionine decarboxylase (*SamDC*) [19]. Homozygous mutants of *SamDC* show a *Minute*-like phenotype including increased developmental time, short and thin bristles and weak wings. In contrast to the phenotype seen in *SamS*/+ derived from maternal *SamS*/*SamS*, the *SamDC*/*SamDC* phenotype is

discrete, i.e. the variability in both phenotype expression and penetrance is low. In this perspective we suggest that the adult external *Minute*-like phenotype seen in *SamS*/+ depleted of maternal *SamS* is caused by an obstructed biosynthesis of polyamines.

The rotated genitalia phenotype seen in males is more difficult to interpret. The adult terminalia, i.e. the anal plate and the genitalia, are developed from the genital disc. The formation of the anal plate, external and internal genitalia take place during the pupal stage [20]. The normal development includes a 360° rotation of the genitalia [21]. The rotated genitalia phenotype is, accordingly, a result of an uncompleted rotation during the pupal stage. A rotated genitalia phenotype has also been seen in homozygous viable alleles of the *Minute* gene encoding ribosomal protein S3 [22]. However, uncompleted rotation of genitalia is seen in exceptional mutant alleles from many genes with different molecular functions. It seems that the development of imaginal disc cells is disturbed by the combination of a maternal lack of *SamS* with a zygotic heterozygosity. Which of these effects can be ascribed to polyamine depletion is still uncertain but since both *SamDC*/*SamDC* and *SamS*/+ derived from *SamS*/*SamS* ovaries show *Minute*-like bristle phenotypes we suggest that at least this phenotype is caused by an impaired polyamine biosynthesis.

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