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Molecular cloning of the *S*-adenosylmethionine synthetase gene in *Drosophila melanogaster*

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Abstract

We have isolated and sequenced cDNA clones encoding the *Drosophila melanogaster S*-adenosylmethionine synthetase. The deduced amino acid sequence contains 405 amino acid residues and shows high homology to rat, yeast, *Arabidopsis* and *Escherichia coli* counterparts. The gene is transcribed throughout *Drosophila* development but its main activity is seen in adult males and females. The highest transcription activity is seen in female ovaries. The transcript has an unusually long 5'-untranslated region, which might be of importance for translational regulation.

Key words: *S*-Adenosylmethionine synthetase; cDNA sequence; Gene expression; 5'-Untranslated region; *Drosophila melanogaster*

1. Introduction

S-Adenosylmethionine synthetase (AdoMet synthetase or ATP:L-methionine *S*-adenosyltransferase (EC 2.5.1.6)), first reported by Cantoni [1], is the enzyme that catalyses the biosynthesis of *S*-adenosylmethionine (AdoMet) by the following reaction [2]:

$$\text{Methionine} + \text{ATP} \xrightarrow[K^+]{Mg^{2+}} \text{AdoMet} + PP_i + P_i$$

AdoMet serves as a cofactor in a variety of reactions. It is the methyl donor in all known methylation reactions except in the methylation of methionine itself. After decarboxylation, AdoMet also serves as a propylamine group donor in the biosynthesis of polyamines. The AdoMet synthetase gene has been studied extensively in bacteria, yeast, plant and animal systems. In *Escherichia coli* the structural gene for the enzyme, MetK, has been cloned and sequenced [3]. Two isozymes, encoded by two different genes (sam-1 and sam-2), have been cloned and sequenced in *Saccharomyces cerevisiae* [4,5]. In mammalian tissues three isoenzymes, α, β, γ, have been identified [6], while in plants the AdoMet synthetase gene has been cloned and sequenced in *Arabidopsis thaliana* [7] and in *Dianthus caryophyllus* [8].

In a study of mutations that act as dominant modifiers of the *zeste–white* interaction in *Drosophila melanogaster*, we have genetically characterized a genomic region on chromosome 2L that most probably includes the gene where the *Suppressor of zeste 5* mutation is localized. This is a gain of function mutation causing a dominant suppression of the orange eye colour of *z* w^{is} males [9]. In a homozygous condition, the mutation is lethal in the embryonic stage. Other modifiers of *zeste*, *Su(z)2* and *E(z)* have been shown to be important for correct transcriptional regulation, not only of the *white* gene expression but also of the homeotic selector genes [10,11]. Using a genomic DNA fragment to which we have mapped the *Su(z)5* gene as a probe, we have screened a *Drosophila* cDNA library in order to find out whether this region was transcribed or not. We could isolate overlapping cDNA clones corresponding to one single mRNA. Sequencing results show that this is the *Drosophila* gene for *S*-adenosylmethionine synthetase.

Here, we present the amino acid sequence of the *Drosophila* AdoMet synthetase deduced from the nucleotide sequence of the cDNA clones. We have compared this amino acid sequence from *Drosophila*AdoMet synthetase to those of rat, *Arabidopsis*, yeast and *E. coli* counterparts. We have also examined the expression of the AdoMet synthetase gene in different developmental stages of *D. melanogaster*.

2. Materials and methods

[α-^{32} P]dCTP, [^{35}S]dATP, [γ-^{32}P]dATP were obtained from Amersham Corp. The DNA Taq Track sequencing kit, enzymes, pGEM-T Vector System, Magic PCR Preps DNA Purification System and sequencing primers were obtained from Promega Corp. Primers used for primer extension were obtained from Symbicom. The *Drosophila* pupae

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The nucleotide sequence has been submitted to the EMBL, Genbank, and DDBJ Nucleotide Sequence Databases with the accession number X77392.

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cDNA library was from Clontech Laboratories. Nitrocellulose membranes were from Schleicher and Schull and Gene Screen Plus membrane filters were from DuPont, NEN Research Products. A Dynal mRNA preparation kit was used. All reagents were of analytical grade. Genomic λ-clones from the cytological region 2L:21A-B in *Drosophila melanogaster* were kindly provided by Dr. Hans-Philippe Lerch, University of Zürich.

2.1. Library screening and DNA sequencing analysis

A pupal cDNA *Drosophila* library (λgt11) was plated on *E. coli* L392 and screened at a density of 20,000 plaques/150 mm plate using a excised fragment from a genomic lambda clone as probe. The inserts in the positive clones were recovered by PCR amplification. After LMT agarose electrophoresis, the PCR products were purified using the Magic PCR Preps DNA Purification System and then ligated into the pGEM-T vector, following the instructions of the manufacturer. With the dideoxy chain termination technique [12], sequencing was performed using the forward, reverse, SP6 and T7 sequencing primers of the pUC/M13 vector. Acrylamide gels (5%) measuring 38 × 50 cm were used. To maximize the number of sequenced nucleotides, samples from each reaction were run for 9, 6 and 2.5 h at constant power, 75 W. All sequences were read at least twice and in both directions, except the 45 starting nucleotides which were read from a genomic clone in reverse direction only.

2.2. mRNA extraction

mRNA was extracted using Dynal biomagnetic separation system. Whole flies, pupae, larvae, embryos or ovaries were frozen in an ethanol/CO_2-ice bath. The frozen tissues were ground in 0.1 M Tris-HCl (pH 8.0); 0.5 M LiCl; 10 mM EDTA; 1% SDS; 5 mM DTT. After this step the instructions of the manufacturer, Dynal, were followed.

2.3. Northern blot analysis

Approximately 1.0 μg of mRNA from different developmental stages were fractionated on a 1.0% formaldehyde-agarose gel as described by Hansson and Lambertsson [13], and blotted onto Gene Screen Plus filters using Vacu Gene Vacuum Blotting System (Pharmacia LKB Biotechnology AB). The filters were pre-hybridized, hybridized and washed following the instructions for Gene Screen Plus filters. The cDNA clone #10 (inserted fragment) and an α-tubulin clone (kindly provided by Prof. A. Lambertsson, Oslo), labelled by the random primer technique, were used as probes.

2.4. Primer extension analysis

The primer extension reaction was performed using 0.6μg of mRNA from adult females as a template. The method described by Göransson et al. [14] was followed using a ^{32}P end-labelled 22mer (nucleotides 92–113) as primer. Samples were run on acrylamide gels (7%) in parallel with a sequence reaction sample containing the same primer and a genomic DNA clone as template.

3. Results and discussion

3.1. Isolation of cDNA clones

From a chromosomal walk, done in Dr. M. Nolls lab (Zürich), we chose to analyze the y36–1 lambda clone further. Since Southern blots of DNA from Su(z)5 mutants showed restriction fragment length polymorphism, we concluded that the *Suppressor of zeste 5* gene was included in y36–1 (results not shown). A 13-kb *Eco*RI fragment in this λ36–1 clone was used as a probe in a screen of a *D. melanogaster* (Canton S) pupal stage cDNA library. Approximately 400,000 clones were screened. We isolated 9 positive clones varying from 0.7 to 2.0 kb in length, see Fig. 1. Following PCR amplification these were subcloned into the pGEM-T vector and analyzed.

3.2. Sequence analysis and primer extension

Analysis of the cDNA clones revealed a transcription unit of 2350 bases. Since no cDNA clone covered the whole transcript, two overlapping clones had to be used to make the complete sequence. We used clones #10 and #12. Fig. 1 shows the extent of the cDNA clones, as well as the complete transcription unit with transcription initiation site, translation start site ATG , the 1215 bp long open reading frame, translation stop site and the existing polyadenylation sites indicated. A primer extension analysis, Fig. 2, showed that none of our cDNA clones included the complete 5′-region. By using the same primer as in the primer extension experiment (nucleotides 92–113), and a genomic subclone from y36–1 as a template, we were able to sequence the 45 bases at the 5′ end that were missing in our cDNA clones. The starting 45 bases in the transcribed sequence are therefore deduced from a genomic clone. As also shown in the primer extension analysis (Fig. 2), there are premature stops for reverse transcriptase at sites +46 and +48, probably due to bad

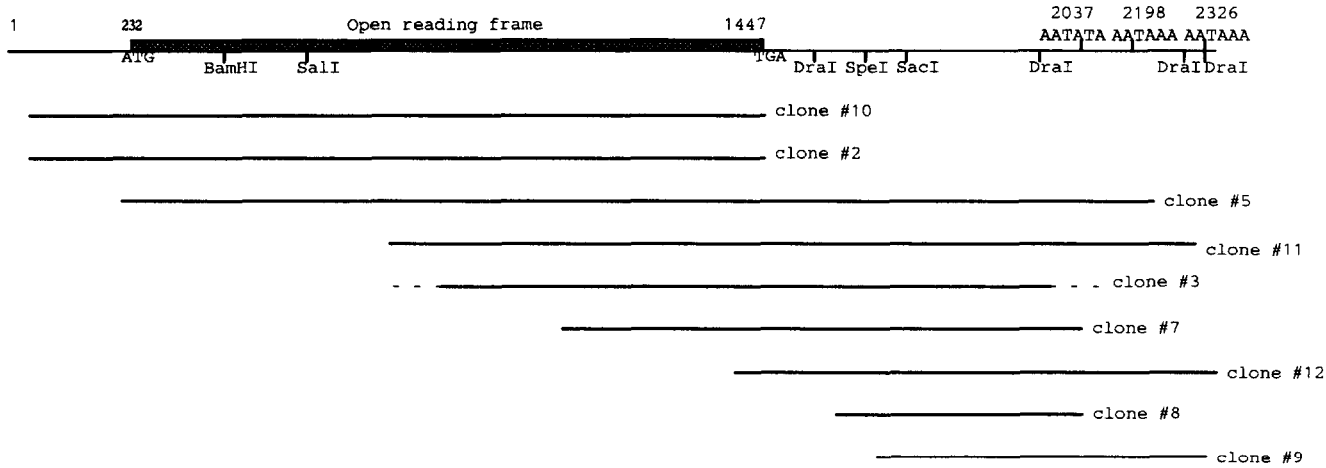

Fig. 1. The *Drosophila melanogaster* S-adenosylmethionine synthetase transcription unit. Organisation of the cDNA clones with restriction sites, transcription start site (1), translation start codon (232–234) and stop codon (1447–1449), ORF and polyadenylation signals are indicated.

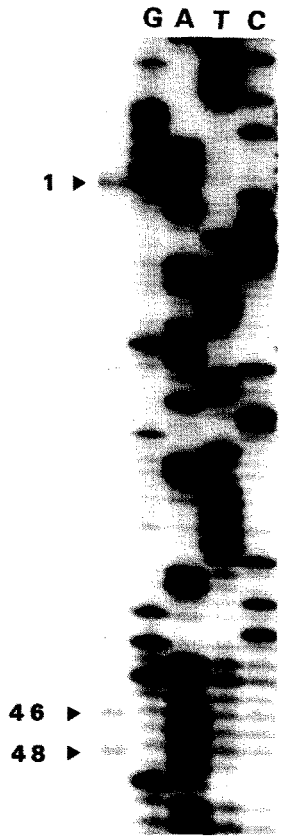

Fig. 2. Primer extension analysis. Poly(A)$^+$ mRNA from Canton S adult females. The primer extension product reveals the transcription start point, +1. Premature transcription stops are seen at positions +46 and +48.

readthrough by the transcriptase. Both clone #2 and clone #10 start at the +46 site. The complete cDNA sequence was run through a computer search to look for homologies with known sequenced genes in the EMBL-database. A strong homology was found to *S*-adenosylmethionine synthetase from different species.

From the primer extension analysis we can conclude that the *Drosophila* AdoMet synthetase gene has a 231 nucleotide-long 5′-untranslated region (5′ UTR). This, however, is not unique to *Drosophila*. The 5′ UTR in AdoMet synthetase mRNAs of murine liver and rat liver are 224 nucleotides [15] and 210 nucleotides [16] long, respectively. AdoMet serves, after decarboxylation, as a propylamino group donor in the synthesis of polyamines. Two other enzymes involved in the biosynthesis of polyamines, *S*-adenosylmethionine decarboxylase (SAMdc) and ornithine decarboxylase (ODC), belong to a specific class of proteins which have leader sequences in their mRNA of more than 200 nucleotides in length [17–19]. These long 5′ UTR in ODC and SAMdc have been proposed to be involved in a feedback translational regulation by polyamines [20]. Even though the sequence of the 5′ UTR in the AdoMet synthetase mRNA is not conserved between *Drosophila* and mammals, the presence of a long 5′ UTR seems to be important for translational regulation of these genes involved in polyamine synthesis. Whether or not polyamine concentration plays a role in the regulation of AdoMet remains to be investigated.

3.3. Amino acid comparison

The deduced amino acid sequence of the *D. melanogaster* AdoMet synthetase is shown in Fig. 3. The first ATG site of the ORF is assumed to be used as the translation start site. The sequence flanking this ATG is TCAACATG which is in good agreement with consensus CCACCATG [21]. The region from Thr^{28} to Gln^{401} in the *Drosophila* amino acid sequence is compared to the homologues in rat kidney, yeast, *Arabidopsis* and *E. coli*. Amino acid identities in aligned sequence are 74% between *Drosophila*/rat, 64% between *Drosophila*/yeast, 56% between *Drosophila*/*Arabidopsis* and 50% between *Drosophila*/*E. coli*. The region that has been proposed to be involved in ATP binding; 145–150 Gly-Ala-Gly-Asp-Gln-Gly and Lys^{174} [22,23], is perfectly conserved through all the species, except in *E. coli* which lacks Lys^{174} (Fig. 3). Two cystein residues have been proposed to be of importance for AdoMet synthetase activity, Cys^{90} in *E. coli* and Cys^{149} in rat kidney [24]. These residues correspond to Cys^{116} and Cys^{163} in Fig. 3. Cys^{116} has been conserved through all the species while Cys^{163} is conserved only in *D. melanogaster*, rat and *E. coli*.

These results indicate that we have isolated and sequenced the *Drosophila melanogaster* AdoMet synthetase gene and that the AdoMet synthetase protein has been well conserved through evolution.

3.4. Northern blot analysis

Poly(A)$^+$ RNA was prepared from different stages in *Drosophila* development and used in Northern blot analysis (Fig. 4). Using a 1.6 kb cDNA fragment from cDNA clone #10 as a probe, the results reveal that two transcripts, approximately 2.0 and 2.3 kb in length, are present in all stages tested. The larger transcript seems to be more abundant in the pupal stage, while the smaller transcript is more abundant in all other stages. The two forms of transcripts could be produced by the use of different polyadenylation signals since there are polyadenylation signal sites at 2198, 2326 (AATAAA) and 2037 (AATATA). The latter deviates from the consensus AATAAA [25], but there is precedence for use of this sequence in other *Drosophila* genes [26,27]. cDNA clones #3, #7 and #8 (Fig. 1), end close to this 2037 AATATA, indicating that this polyadenylation site is being used. This polyadenylation signal would produce a transcript of about 2.0 kb, compared to 2.3 kb using the 2326 AATAAA signal, used in clones #2 and #10. Results from Southern blot analysis reveals that, in contrast to yeast, *Arabidopsis* and mammals, AdoMet synthetase in *Drosophila* is encoded by one unique gene (results not

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   1                                                                    70
D  MPQKTNGHSA NGCNGSNGNS YDMEDGATFL FTSVSVGEGH PDKMCDQISD AILDAHLKQD PNAKVACETV
RK     M**Q-- -----L**FH EAFIEEG*** ***E****** ***I****** *V*****Q** *D********
S                          MAG*** ***E****** ***I***V** *****C*AE* *HS******A
A                            ME*** ***E**N*** ***L****** *V***C*E** *DS******C
E                            MAKH* ***E**S*** ***IA***** *V***I*E** *K*R*****Y

71                                                                  140
AKTGMILLC- GEITSKAVVD YQKVIRETVQ HIGYDDSSKG FDFKTCNVLL ALDQQSPEIA AGVHVN--RA
********A- *****R*AI* ****V**AIK ********** **Y******V **E****D** Q***LD--*N
******MVF- ****T**QL* ***IV*D*IK K******A** **Y******V *IE****D** Q***EE--KD
T**N*VMVF- ****T**T** *E*IV*D*CR A**FVSDDV* L*ADK*K**V NIE****D** Q***GHFTKC
V***IGFSWR RNHHQRPW** IEEIT*N**R E***VH*DM* **ANS*A**S *IGK***D*N Q**DRA---D

141                                                                 210
EEEIGAGDQG IMFGYATDET EEC-MPLTVV LAHKLNEKIA ELRRSDVFWW ARPDSKTQVT CEYLFNQGSA
**D******* L********* ***-****I* ******A*L* ****NGTLP* L********* VQ*MQDR*AV
L*D******* ********** P*G-L***IL ******MAM* DA**DGSLA* L***T***** V**KDDH*RW
P*D******* H********* P*L-***SH* **T**GARLT *V*KNGTCA* L***G***** V**YNDK*AM
PL*Q****** LDVSATQLMK PT*L**APIT Y**R*VQRQ* *V*KNGTL-R V***A*S*** FS*--DD*KI

211                                                                 280
VPKRVHTIVV SMQHSEKISL ETLRSEVMEK VVKVVIPAKY IDANTIVHIN PCGLFVIGGP MGDAGLTGRK
I*I******I *V**D*EVC* DEM*DALK** LI*A*V**** L*ED**Y*LQ *S*R****** Q*********
**Q*ID*V** *A**ADE*TT *D**AQLKSE IIEK***RDM L*E**KYF*Q *S*R****** Q**T******
**I****VLI *T**D*TVTN DEIARDLK*H *I*P***E** L*EK**F*L* *S*R****** H*********
*G--IDAV*L *T****E*DQ KS*QEA***E II*PIL**EW LTSA*KFF** *T*R****** ***C******

281                                                                 350
IIVDTYGGWG AHGAGAFSGK DFTKVDRSAA YAARWVAKSL VKAGLCKRCL VQVSYAIGLA EPLSITVFDY
********** ***G****** *Y******** ********** **G***R*V* ********VS H****SI*H*
****A***AS SV*G****** *YS******* ********** *A******VQ **F*****I* ****LH*DT*
**I******* ***G****** *P******G* *IV*QA***V *AN*MAR*A* ********VP ****VF*DT*
*****T**MA R**G****** *PS******* ****Y***NI *A***AD**E I********* **T**M*ETF

351                                                          400          423
GTSHKSQKEL LDIIRRNFDL RPGKIVKDLN LRQ---PIYQ RTSTYGHFGR A--GFSWESQ ASGD
***Q**ER** *E*VKN**** ***V**R**D *KK---**** **AA****** D--S*P**VP KKLKY
**AT**DE*I I***SK**** ***VL**E*D *AR---*F*L P*AS****TN Q--EYP**KP KTLKF
E*GLIPD**I *K*VKES**F ***MMTIN*D *KRGGNGRFL K*AA****** DDPD*T**VV KPLKWDKPQA
**EKVPSEQ* TLLV*EF*** PI*L*----Q MLDLLH***K E*AA****** E--H*P**KT DKAQLLRDAA GLK
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Fig. 3. Comparison of the deduced amino acid sequence of *D. melanogaster* (D) *S*-adenosylmethionine synthetase with those of rat kidney (RK), *S. cerevisiae* (S), *A. thaliana* (A) and *E. coli* (E). Amino acids are represented by the single-letter code. The sequences are aligned for maximum similarity. Identical amino acids are indicated by stars and gaps by dashes. The sequence data used have the following accession numbers in the EMBL databank; RK; P18298, S; P10659, A; P23686, E; P04384

shown). The gene is most active in adult flies, and a comparison of intact females, females where the ovaries have been removed, and ovaries separately, indicates that the main activity is within ovaries (Fig. 4). AdoMet is needed in all living cells as a methyl group donor. Therefore, the AdoMet synthetase gene could be considered to be a housekeeping gene. Most housekeeping genes are expected to be expressed constitutively. This is also seen with the *Drosophila* AdoMet synthetase gene, since the gene is expressed throughout all tested stages (Fig. 4). However, even though the gene is expressed constitutively, a pronounced stage and tissue specific expression is seen with a high activity in the ovaries. Tissue specific expression of AdoMet synthetase has also been demonstrated in *Arabidopsis* [7].

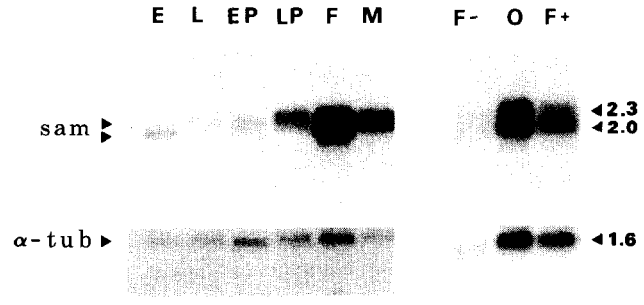

Fig. 4. Northern blot analysis. Poly(A)$^+$ mRNA from embryo (E), third instar larvae (L), early pupae (EP), late pupae (LP), adult females (F), adult males (M), female without ovaries (F−), ovaries (O) and intact females (F+). The filters were hybridized with cDNA clone #10 as a probe, (sam). The α-tubulin (α-tub) probe was used to verify the amounts of RNA loaded in each lane.

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