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The emerging role of adenosine deaminases in insects

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Abstract

Adenosine deaminases catalyze the deamination of adenosine and deoxyadenosine into their respective inosine nucleosides. Recent sequencing of the genomes of several model organisms and human reveal that Metazoa usually have more than one adenosine deaminase gene. A deficiency in the gene encoding the major enzyme is lethal in mouse and *Drosophila* and leads to severe combined deficiency (SCID) in human. In these organisms, enzyme deficiency causes increased adenosine/deoxyadenosine concentration in body fluids and some organs. Elevated levels of adenosine and deoxyadenosine are toxic to certain mammalian and insect cells, and it was shown for human and mouse that it is a primary cause of pathophysiological effects. Data suggest that the major role of adenosine deaminases in various taxa is the protection of tissues against increased levels of adenosine and deoxyadenosine. This review also discusses potential roles of adenosine deaminases in *Drosophila* metamorphosis and the employment of a *Drosophila* model to study the cell-specific toxicity of elevated nucleoside levels.
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1. Adenosine deaminases are found in all organisms

Adenosine deaminases are metabolic enzymes involved in the enzymatic conversion of the nucleosides adenosine and 2′-deoxyadenosine (referred to as deoxyadenosine) into inosine and 2′-deoxyinosine, respectively (reviewed in Hershfield and Mitchell, 2001). Adenosine deaminases are evolutionarily conserved proteins with a $(\beta\alpha)_8$ barrel structure and zinc atom in the catalytic pocket. The X-ray structure of mouse adenosine deaminase (ADA) provided detailed knowledge of the molecular conformation of the active site, thus further elucidating its catalytic mechanism (Wilson et al., 1991). Alignment of various adenosine deaminases is shown in Fig. 1. It is clear that the essential residues in the ADA active site are retained in all organisms from *Escherichia coli* to human (Chang et al., 1991).

Recent discoveries and the completion of genome sequencing of several model organisms disclosed the existence of two subfamilies of adenosine deaminases in Metazoa. Most species carefully examined, including *Drosophila melanogaster* and human, contain genes of both subfamilies (Table 1). Phylogenetic analysis of various ADAs performed by parsimony, maximum likelihood or neighbor-joining algorithms can clearly distinguish both major evolutionary branches. Fig. 2 shows an example of a tree generated by the maximum parsimony analysis. To see the distance from the related AMP deaminases, the *Drosophila* sequence (GenBank accession no. AI113954) is also included.

Members of the first subfamily, called *bona fide* adenosine deaminases, are metabolic enzymes that have been studied for more than three decades. They are characterized by a relatively short N-terminal region and predominantly cytoplasmic localization. Proteins belonging to this subfamily have been discovered in all organisms that have been investigated, including

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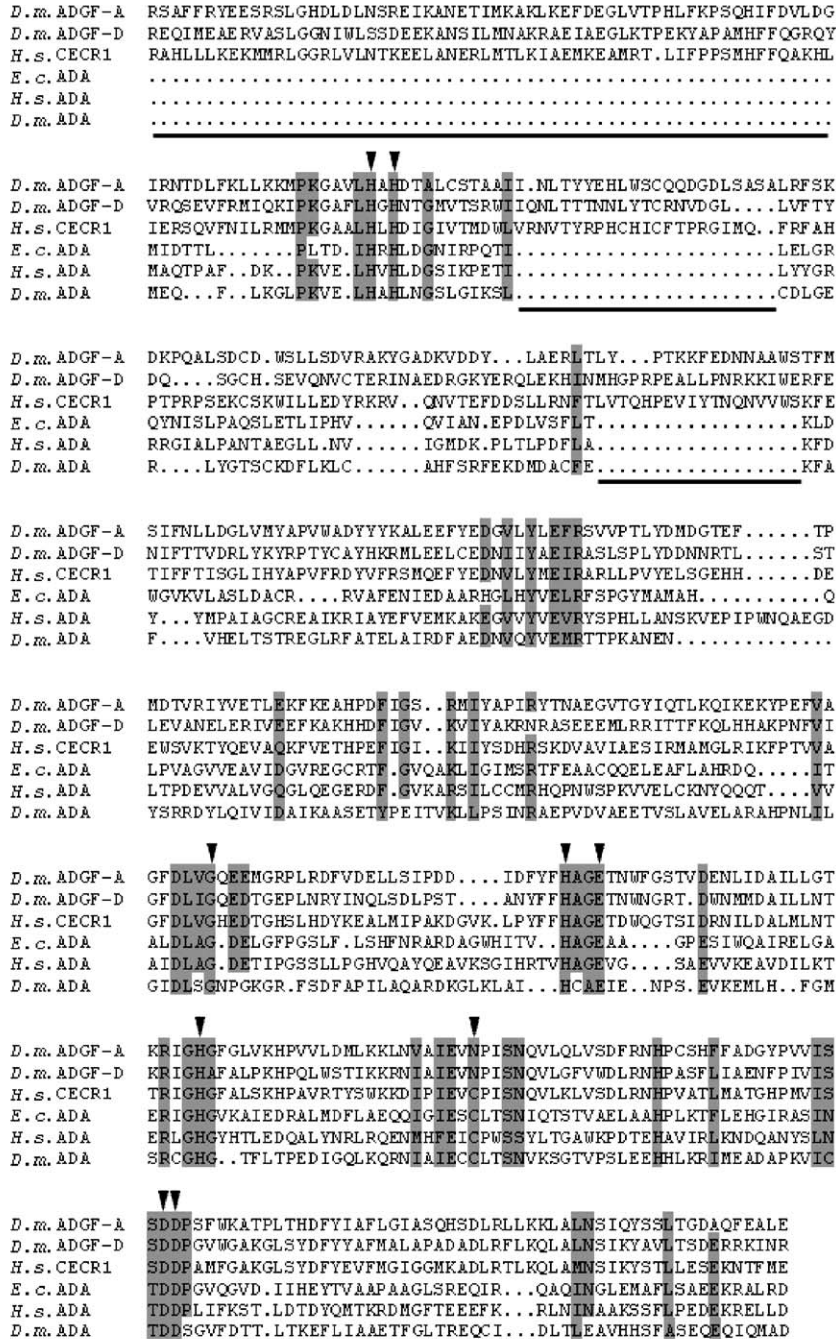

Fig. 1. Comparison of six adenosine deaminases representing two major subclasses. The ADGF/CECR1 subfamily is represented by *Drosophila* ADGFs (D.m. ADGF-A and ADGF-D) and human CECR1 (H.s. CECR1). The *bona fide* subfamily is represented by *E. coli* ADA (E.c. ADA), human ADA (H.s. ADA) and *D. melanogaster* ADA (D.m. ADA). Arrows indicate conserved amino acids located in the active site of the mammalian enzyme (Wilson et al.,1991) box-shaded AA—conserved amino acids mostly making short conserved domains. The portions of ADGF-A and –D and human CECR1 containing signal peptides as well as the very C-terminal sequences are not shown. Solid lines mark the N-terminal portions of the ADGF/CECR molecules missing in *bona fide* ADAs. GenBank accession number of *Escherichia coli* ADA is NP_416140. The accession numbers of other genes are listed in Table 1.

human, mouse, *D. melanogaster*, *C. elegans* and *S. cerevisiae.* The single *Drosophila* gene of this subfamily can be found by BLAST search in the genomic database, but has not been characterized thoroughly. The homologous human gene was associated with combined immunodeficiency—SCID (Giblett et al., 1972). It was localized on the long arm of chromosome 20 (at 20q12-q13.1) (Tischfield et al., 1974; Mohandas et al., 1980, 1984) and the corresponding cDNA was sequenced (Adrian et al., 1984; Daddona et al., 1984; Valerio et al.,

Table 1
List of adenosine deaminase-like genes in *Drosophila melanogaster*, *Anopheles gambiae*, human, mouse, *Culex pipiens quinquefasciatus*, *Aedes aegypti*, *Sarcophaga peregrina*, *Escherichia coli*, *Apis melifera*, *Glossina morsitans*, *Dictyostelium discoideum*, *Lutzomyia longipalpis* and *Aplysia californica*

Organism	Gene	Enzyme subclass	GenBank
D. melanogaster	*D.m.* ADA	I	NM_141609
	D.m. ADGF-A	II	NM_079406
	D.m. ADGF-A2 (MSI)	II	NM_080281
	D.m. ADGF-B	II	AF384215
	D.m. ADGF-C	II	AF337552
	D.m. ADGF-D	II	AF337553
	D.m. ADGF-E	II	NM 137133
A. gambiae	*A.g.* ADA	I	XP 314048
	A.g. ADA2	II	EAL41919
	A.g. ADA3	II	XP 308848
	A.g. ADA4	II	XP 309067
H. sapiens	*H.s.* ADA	I	BC040226
	H.s. ADA-like	I	XM 091156
	H.s. CECR1	II	BC051755
M. musculus	*M.m.* ADA	I	NM 007398
	M.m. ADA-like	I	AAH50879
			BC050879
C. pipiens	*C.p.* ADA	II	AF298886
A. aegypti	*A.e.* ADA	II	AAL76033
S. peregrina	*S.p.* IDGF	II	D83125
E. coli	*E.c.* ADA	I	NP_416140
A. melifera	*A.m.* ADA	I	XP_394309
	A.m. ADGF-like	II	XP_392577
G. morsitans	*G.m.* TSGF1	II	AAD52851
	G.m. TSGF1	II	AAD52850
D. discoideum	*D.d.* CECR1-like	II	AAO52254
L. longipalpis	*L.l.* LuloADA	II	AAF78901
A. californica	*A.c.* MDGF	II	AAD13112

1984; Wiginton et al., 1984). The sequencing of the human genome revealed another gene encoding ADA-like protein on chromosome 15 (at 15q15.1). This gene has not been characterized but several clones can be found in human EST databases. Both human ADA genes represent distantly related members of the first subfamily and their open reading frames encode proteins with 19% identity.

Members of the second ADA subfamily, called ADGF/CECR1, were discovered only recently. They differ from the *bona fide* enzymes because of an extended N-terminus. These termini often contain a signal peptide for specific cellular localization or secretion (Fig. 2). Several proteins of this subfamily have been shown to posses adenosine deaminase enzymatic activity (Charlab et al., 2001; Zurovec et al., 2001) and to be mitogenic in vitro (Homma et al., 1996; Zurovec et al., 2001). They have been found in flesh fly *Sarcophaga peregrina* (Homma et al., 1996), sand fly *Lutzomyia longipalpis* (Charlab et al., 2000), *Drosophila* (Maier et al., 2001), the sea hare mollusk *Aplysia californica* (Sossin et al., 1989; Akalal and Nagle, 2001) and human (Riazi et al., 2000). A homologous EST was detected in the slime mold *Dictyostelium discoideum* (Maier et al., 2001). No genes of ADGF/CECR1 have been detected in the completed sequences of mouse and *C. elegans* genomes. The single human gene assigned to this subfamily is called CECR1 (Cat Eye Critical Region 1). It is localized on chromosome 22 and its duplication was implicated in the genetic disease cat eye syndrome (CES) (Riazi et al., 2000).

The founding member of the ADGF/CECR1 subfamily was isolated from conditioned media of flesh fly (*S. peregrina*) embryonic cells (NIH-SAPE-4) and was called insect-derived growth factor (IDGF; Homma et al., 1996; Matsushita et al., 2000). Since the name IDGF was being used previously for a family of unrelated *Drosophila* growth factors (Kawamura et al., 1999), the *Drosophila* homologs of *Sarcophaga* ADA were designated adenosine deaminase-like growth factors (ADGFs). Our homology search in the complete sequence of *Drosophila* genome revealed six related ADGF genes. One of them, ADGF-A2, was described by Matsushita et al. (2000) as male-specific IDGF (MSI; Table 1).

Comparison of various organisms suggests that higher Diptera contain an unusually high number of ADA genes (Table 1). Six ADGF genes and one *bona fide* ADA were detected in the *Drosophila* genome. ADGF-like genes were found in several other Diptera, including the sand fly (Charlab et al., 2000), tsetse fly (Li and Aksoy, 2000), and the mosquitoes *Culex quinquefascia-*

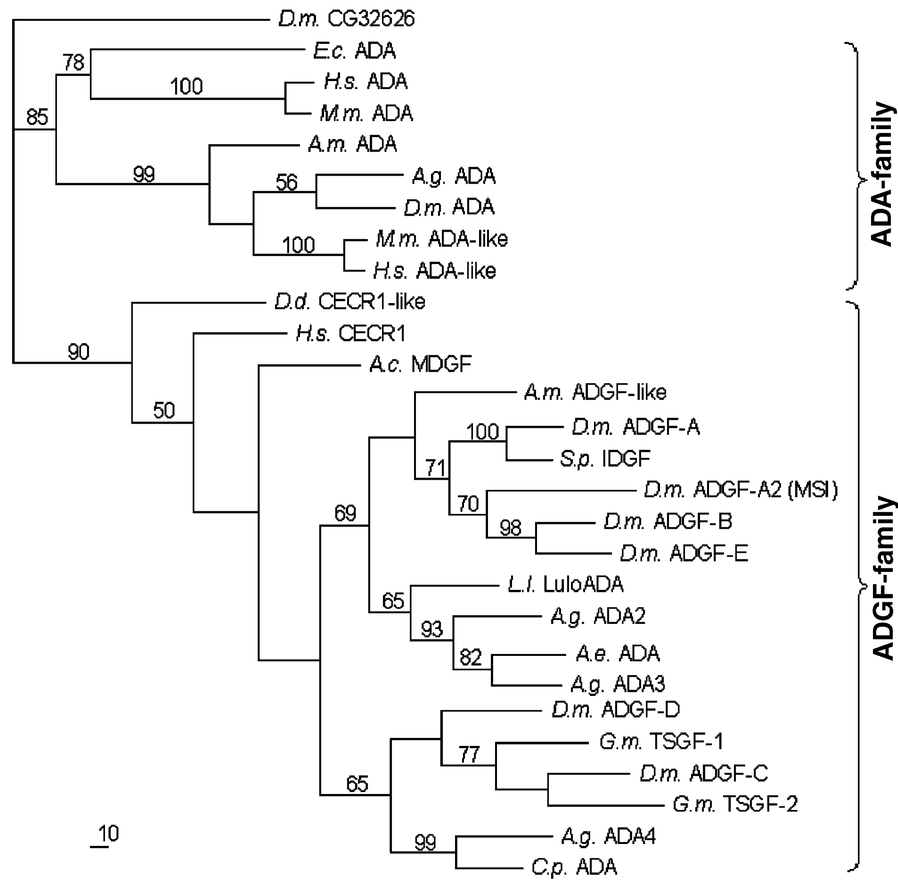

Fig. 2. Maximum parsimony phylogenetic tree as inferred from amino acid sequences. Parsimony analysis was performed using PAUP* 4.0 beta version (Swofford, 1998) with branch-and-bound search, when ambiguous alignment regions were excluded from analyses. Bootstrap values >50% (obtained from 500 replicates). First two letters in the gene name designate the organism: *A.g.* (*Anopheles gambiae*), *A.m.* (*Apis melifera*), *A.c.* (*Aplysia californica*), *C.p.* (*Culex pipiens*), *D.d.* (*Dictyostelium discoideum*), *D.m.* (*Drosophila melanogaster*), *E.c.* (*Escherichia coli*), *G.m.* (*Glossina morsitans*), *H.s.* (*Homo sapiens*), *L.l.* (*Lutzomyia longipalpis*), *M.m.* (*Mus musculus*) *and S.p.* (*Sarcophaga peregrina*). CG32626 is *Drosophila* AMP deaminase (GenBank accession no. AI113954). GenBank accession numbers of other genes are listed in Table 1.

tus and *Aedes aegypti* (Ribeiro et al., 2001). The established gene sequences are incomplete in most species, but available information is sufficient for phylogenetic analysis. Maier et al. (2001) constructed a gene tree of adenosine deaminases consistently with current concepts of the phylogenetic relationships among sampled organisms and concluded that four gene duplications occurred in the evolution of Diptera. Two duplications seem to be associated with early phylogeny of the order, as indicated by the presence of four ADA genes in the completely sequenced mosquito genome of *Anopheles gambiae*. Further duplications probably followed only in the evolutionary branch of cyclorrhaphous Diptera.

2. Effect of adenosine deaminase, adenosine and deoxyadenosine on cell growth in vitro

Several members of the ADGF/CECR subfamily have been implicated in the control of cell growth (Homma et al., 1996; Charlab et al., 2001; Maier et al., 2001). It was suggested that *Sarcophaga* IDGF binds to the specific cell surface receptor, which transmits the proliferative signal into NIH-SAPE-4 cells (Homma et al., 2001). We did not detect any specific binding of ADGF-A and –D to *Drosophila* cells (Zurovec, unpublished). We found that the adenosine deaminase enzymatic activity of ADGF-A and –D is essential for their mitogenic effect on *Drosophila* S2 embryonic cells, *Drosophila* Cl8+ imaginal disc cells and *Sarcophaga* NIH-SAPE-4 cells (Zurovec et al., 2001, 2002). The mitogenic effect of ADGFs on Cl8+ and S2 cells can be mimicked by depletion of adenosine from the culture media. This could be done by the addition of bovine ADA to the standard medium or by using media prepared without adenosine. Recombinant ADGFs exhibited no growth effects on cells under adenosine-free conditions. We conclude that the growth stimulation of cultured cells by ADGFs is due to a reduction or a depletion of adenosine and deoxyadenosine from the culture medium (Zurovec et al., 2002).

The inhibition of vertebrate and invertebrate cell growth by adenosine and deoxyadenosine was observed earlier (Henderson and Scott, 1980; Henderson et al., 1980). Most information comes from studies in mammalian tissue cultures. It was concluded that adenosine affects mammalian cells in two different ways. At low concentrations (nanomolar and micromolar), adenosine exerts its physiological effects (positive or negative) via activation of specific cell surface receptors, whereas it produces toxic effects at high doses (millimolar concentration), probably by interfering with homeostasis of the intracellular nucleotide pool (Schrier et al., 2001; Merighi et al., 2002). Low concentrations (1–10 μM) of adenosine were reported to be mitogenic on some endothelial cells (Meininger et al., 1988; Ethier et al., 1993), mesangial cells (MacLaughlin et al., 1997) and microglial cells (Gebicke-Haerter et al., 1996). In contrast, such adenosine concentrations can negatively influence growth of human arterial smooth muscle cells and significantly increase the number of apoptotic nuclei (Peyot et al., 2000).

The toxic threshold of adenosine varies depending on cell type. For human macrophages, it is toxic at 3 μM or above (Hasko et al., 2000), whereas cell divisions in a murine neuroblastoma cell line are blocked by 100 μM adenosine (Schrier et al., 2001). A higher adenosine concentration (100 μM) was shown to induce apoptosis in endothelial cells (Ethier et al., 1993). Many mammalian cell types, including lymphocytes, are resistant to adenosine due to the endogenous production of a high level of ADAs. When ADA function is blocked by mutation or its specific inhibitor coformycine, adenosine is usually toxic at 5–50 μM (Hershfield and Mitchell, 2001).

The other ADA substrate, deoxyadenosine, when added to growth media at higher concentration is also toxic to various mammalian cells, including lymphocytes, because it affects dNTP homeostasis. Increased deoxyadenosine level can also induce apoptosis in the lymphoblasts (for a review, see Hershfield and Mitchell, 2001).

The effects of adenosine and deoxyadenosine on insect cells in vitro are reminiscent of those observed in mammalian tissue cultures. Adenosine and deoxyadenosine concentrations exceeding 10 μM are toxic at *Drosophila* cell lines S2 and Cl8+, *Sarcophaga* NIH-SAPE-4 (Zurovec et al., 2002), and *Aedes* C-7 (Sherwood and Stollar, 1982). In contrast, *Drosophila* neuroblast cell line Bcg-c6, haematopoietic line Mbn-2 and embryonic line Kc167 are resistant to 100 μM of adenosine and deoxyadenosine. We analyzed endogenous adenosine deaminase activities in the cell lysates of several *Drosophila* cell lines and found a correlation between cell survival and adenosine concentration. The mechanism of the adenosine resistance is similar to that of mammalian cells and rests on the high activity of endogenous adenosine deaminases (Zurovec et al., 2002; Table 2).

Table 2
Survival in the presence of 100 μM adenosine and endogenous adenosine deaminase activity for five different *Drosophila* cell lines (Zurovec et al., 2002)

Cells	Toxicity of 100 μM adenosine	Endogenous ADA activity in cell extract (U/mg)
Cl.8+	+	0.011 ± 0.003
S2	+	0.006 ± 0.003
Bcg-c6	–	0.065 ± 0.009
Mbn-2	–	0.035 ± 0.004
Kc167	–	0.020 ± 0.011

The experiments with in vitro treatments of mammalian and insect cells revealed that different cell types vary considerably in their sensitivity to increased adenosine/deoxyadenosine concentrations. Endogenous adenosine deaminases are responsible for the protection of cells to these nucleotides.

3. Expression of adenosine deaminases in organisms in vivo

Initial reports on ADA activity in insect tissues came from the early research of purine catabolism in *Drosophila* (Hodge and Glassman, 1967). *Drosophila* mutations resistant to purine were selected on media supplemented with purine and aminopurine. One of the mutants developed purine resistance by elevating adenosine deaminase activity (Dutton and Chovnick, 1990).

More recently, adenosine deaminase activity was detected in the salivary glands of the blood sucking adult Diptera: the mosquitoes *Culex pipiens quinquefasciatus* and *A. aegypti* (Ribeiro et al., 2000), tsetse fly *Glossina m. morsitans* (Li and Aksoy, 2000) and sand fly *Lutzomiya longipalpis* (Charlab et al., 2000). It was suggested that ADA activity represents an adaptation to blood feeding because degradation of adenosine at the bite site might mitigate the perception of pain (Li and Aksoy, 2000; Charlab et al., 2000). Transcripts of *Sarcophaga* IDGF were detected in early embryos and first instar larvae (Homma et al., 2001).

More thorough studies on the expression of ADAs have been performed in *Drosophila* (Maier et al., 2001; Zurovec et al., 2002). Northern blotting and in situ hybridization to RNA revealed that the majority of fruit fly adenosine deaminases are produced by the genes of *ADGF/CECR1* subfamily and the single ADA gene from the *bona fide* subfamily shows only a low level of ubiquitous expression. ADGF-A is the most abundantly expressed ADGF isoform in both larvae and adults. It is expressed strongly in the gut and lymph glands (Zurovec

et al., 2002) and weakly in imaginal discs (unpublished), suggesting the importance of control of local adenosine/deoxyadenosine concentrations in vivo. ADGF-D is the second most highly expressed homologue, with the highest levels in the fat body and brain and peak expression in adults. ADGF-C shows a low level of male-predominant transcription with the expression pattern at least partly overlapping with that of ADGF-D. ADGF-B and –E and ADGF-A2 are expressed only in male and transcription of the three genes starts in pupal stage. The male-specific expression of the three ADGF family members suggests that testis requires special protection against adenosine/deoxyadenosine.

Human ADA activity is found in all tissues examined, but the levels vary over 10^3–10^4 range. The highest activity is found in the guts, lymph nodes and thymus (Hershfield and Mitchell, 2001). Research on ADA-SCID (severe combined immunodeficiency) patients revealed that most adenosine deaminase activity can be ascribed to the *bona fide* ADA from chromosome 20. Other sources of ADA activity accounted for only 1–2% of the total, as revealed by comparison of ADA activity in blood plasma of ADA-SCID patients and a control group (Daddona and Kelley, 1981). It is not clear whether this activity was linked to the ADA-like gene on chromosome 15 or to CECR1 on chromosome 22. No information has been published on the expression of the ADA-like gene on chromosome 15, except for its presence in placental, brain, prostate and cervix-specific EST clones. The expression of the CECR1 gene has been examined by northern blotting and the highest levels were detected in the placenta and embryonic heart, lungs and kidneys (Riazi et al., 2000).

High accumulation of ADA enzymatic activity in spleen, thymus and alimentary tract was also observed in other mammalian species, including mouse, rat, guinea pig, rabbit, cat, dog and calf (Brady and O'Donovan, 1965; Chechik et al., 1983). In mice, rats, cats and guinea pigs, high ADA expression was also found in the placenta (Brady and O'Donovan, 1965; Migchielsen et al., 1996).

The distribution of the peaks in adenosine deaminase activity in mammals shows an interesting parallel to the maximal levels of ADGF-A expression in *Drosophila*. Hence, mammals and insects may use ADAs to similar ends but employ different subfamily members as the principal enzyme. The most conspicuous tissue with highest enzymatic activity in both groups of organisms is gut and lymphoid organs and/or blood cells. Expression in gut is probably an important barrier against surges of adenosine from food (Conway and Cooke, 1939). Alternatively, a high level of purine degradation may be involved in defense against parasites (Witte et al., 1991). It has been suggested that the blood cells regulate adenosine/deoxyadenosine level in blood (Wintrobe, 1959; Giblett, 1985). Because *Drosophila bona fide* ADA has very low level of expression and appears to have accumulated mutations blocking its catalytic activity, it seems likely that the ADGF family members have replaced this function. Thus ADGFs could have evolved an expression pattern that best replaces the required ADA functions.

4. Adenosine deaminase deficiency is lethal in *Drosophila* and in mouse

Genetic analysis of *Drosophila* ADGFs reveals that at least some of these proteins are essential for the life of flies. The loss of function of the most prominent *Drosophila* adenosine deaminase, ADGF-A, leads to death of larvae phenotype (Dolezal et al., 2003). Mutant larvae show disintegration of the fat body and develop melanotic tumors. Since ADGF-A expression is particularly strong in embryos and larvae, early lethality is not surprising. The phenotypes of ADGF-C and ADGF-D mutants are manifested at later stages and are cumulative in double mutants. They include slow movements, lethargy and low fecundity in adults. Defects in ADGF-A2 and ADGF-B do not lead to obvious phenotype, possibly because they are functionally redundant with ADGF-E (Dolezal et al., 2003). Mutants of ADGF-E or *bona fide* ADA have not been reported.

The *Drosophila* larvae homozygous for the ADGF-A^- show a significant increase of adenosine and deoxyadenosine in the haemolymph (Table 3A). They can be rescued by ectopic ADGF-A expression using a heat-shock or UAS promoter (driven by actin-Gal4 driver; Dolezal et al., 2003). The rescue of ADGF-A mutants depends on the dose of ectopic expression. This suggests that the pathological effect of the mutation might be caused by cell-specific sensitivity of certain vital tissue(s) to elevated adenosine/deoxyadenosine.

Defects in human ADA are deleterious for lymphocytes and lead to SCID (Hershfield and Mitchell, 2001). Although the selectivity of ADA^- for lymphocytes is currently not understood it is clear that all the defects in SCID result from increased concentrations of ADA substrates (Hirschhorn, 1999). ADA-SCID patients show two orders of magnitude higher adenosine and deoxyadenosine concentrations in the blood than healthy individuals (Kuttesch et al., 1978). Injection of patients with exogenous ADA decreases nucleoside concentration in their blood and restores functional immunity (Hershfield and Mitchell, 2001). The principal role of human ADA therefore seems to be the protection of tissues against adenosine/deoxyadenosine toxicity.

The major biochemical mechanism of lymphotoxicity is believed to be elevated deoxyadenosine (Table 3B), which leads to the intracellular accumulation of toxic

Table 3
(A) Concentration of adenosine and deoxyadenosine in the haemolymph of ADGF-A mutant and control (wild-type) *Drosophila* larvae. (B) Data from human: adenosine and deoxyadenosine concentration in plasma or serum of ADA-deficient patients and controls (based on the data from Hershfield and Mitchell, 2001)

(A) *D. melanogaster*	Adenosine level (μM)	Deoxyadenosine level (μM)
ADGF-A mutant	0.8–1.9	0.25–1.7
Control	0.06–0.3	Undetected
(B) Human	Adenosine level (μM)	Deoxyadenosine level (μM)
ADA deficiency	<0.1–10	0.1–7
Control	<0.05–0.4	Undetected

Larval haemolymph was collected from several larvae and centrifuged to pellet the blood cells. Each sample was analyzed by liquid chromatography on a 150 mm × 1 mm Synergy Hydro RP column and peaks of adenosine and deoxyadenosine were detected by electrospray tandem mass spectrometry of their respective product ion m/z 136 arising by the collisionally induced cleavage of the nucleotide sugar moiety. The wild-type concentration of dAdo was below detection limits (Dolezal et al., submitted).

dATP. There is a higher deoxyadenosine concentration in thymus, because it is a site of massive cell death due to the differentiation of lymphocytes (Hirschhorn, 1999). Other proposed mechanisms involve interactions of elevated adenosine (Table 3B) with adenosine receptors on lymphocytes, leading to apoptosis (Hershfield and Mitchell, 2001).

The role of additional human genes with potential adenosine deaminase activity in the metabolism of both nucleosides is not known. It is possible that their functions overlap to some extent like the functions of *Drosophila* ADGFs. Such a model might explain why in ADA background lymphocytes are more sensitive to increased adenosine/deoxyadenosine concentration than other cells. Lymphocytes might simply have a low level of ADA-like or CECR proteins, which would in ADA^- conditions lead to the complete loss of protection against adenosine/deoxyadenosine.

ADA-deficient mouse was generated by two groups, resulting in animals with independent sites of *Ada* gene disruption (Wakamiya et al., 1995; Migchielsen et al., 1995). In each case a similar phenotype was observed. ADA-knockout mice fetuses die around birth, possibly as a consequence of severe liver impairment (Wakamiya et al., 1995; Migchielsen et al., 1995). In addition, the lungs of ADA-deficient fetuses are abnormal (Blackburn and Zhong, 2001). An increased concentration of deoxyadenosine was detected in the placenta of these fetuses due to a high apoptotic rate. In the case of ectopic placental ADA expression (ADA-deficient mice carrying a trophoblast-specific ADA minigene), young mice develop quite normally, but display a combined immunodeficiency together with severe lung inflammation, and dying early after birth. (Wakamiya et al., 1995; Migchielsen et al., 1995). In conditions of low ADA expression an elevated concentration of deoxyadenosine and its phosphorylation product dATP was detected in thymus (Migchielsen et al., 1996). Knockout of the ADA gene in mouse revealed that ADA deficiency is less selective and more severe in mouse than in man (Hershfield and Mitchell, 2001). This result reflects differences between both species in purine metabolism in lymphoid and organ development. It may also be important that the CECR1 gene is missing in the mouse genome.

Adenosine deaminase deficiency in human, mouse and *Drosophila* leads to increased adenosine/deoxyadenosine concentration in body fluids and some organs. Elevated concentration of those nucleosides is toxic to certain cell types. The high concentration of ADA substrates in ADA-deficient human and mouse is a primary cause of pathophysiologic effects. Consistently, our data on *Drosophila* ADGF mutants suggest that the toxic levels of nucleosides play key roles in the establishment of mutant phenotypes.

5. Conclusions

Adenosine deaminases catalyze the irreversible, hydrolytic deamination of adenosine and deoxyadenosine. The absence of this enzyme in various organisms results in accumulation of both substrates. Adenosine is a component of adenine nucleotides including ATP and can be made both from the breakdown of ATP and from degradation of RNA following cell death (Hirschhorn, 1999). Deoxyadenosine is primarily derived from the breakdown of DNA at sites of cell death (Chan, 1979; Hirschhorn, 1999).

The accumulation of adenosine and deoxyadenosine in mouse and human plasma or in *Drosophila* haemolymph is toxic to certain cell types and leads to severe phenotypes, including immunodeficiency and even lethality. The phenotypes of adenosine deaminase-deficient mutants are dose-dependent, suggesting that local regulation of extracellular adenosine/deoxyadenosine and protection of tissues against increased concentration of these nucleosides play critical roles in development.

Drosophila and other higher Diptera have more adenosine deaminase genes than other insects or Metazoa. The expression of most of the *Drosophila* adenosine deaminases (ADGF-A2,-B,-C and E) starts in later larval development and the pupal stage, suggesting that these enzymes play some role(s) during metamorphosis. The metamorphosis of *Drosophila* and other higher Diptera involves massive apoptosis and tissue remodeling (Heming, 2003). Most of the specifically

larval tissues, such as salivary glands and epidermis, are made of polyploid cells that are destroyed and replaced by new cells. The resulting cell death produces adenosine and deoxyadenosine in the haemolymph. The elaborate expression pattern of adenosine deaminases may provide protection for growing tissues (including the male germ line and imaginal discs) against the nucleosides. It is therefore possible that the duplication of ADGF genes evolved in higher Diptera in association with profound tissue reconstruction during metamorphosis.

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References

Adrian, G.S., Wiginton, D.A., Hutton, J.J., 1984. Characterization of normal and mutant adenosine deaminase messenger RNAs by translation and hybridization to a cDNA probe. Hum. Genet. 68, 169–172.

Akalal, D.-B.G., Nagle, G.T., 2001. Mollusk-derived growth factor: cloning and developmental expression in the central nervous system and reproductive tract of Aplysia. Mol. Brain Res. 91, 163–168.

Blackburn, M.R., Zhong, H., 2001. Adenosine deaminase-deficient mice as models for adenosine-mediated lung inflammation and damage. Drug Dev. Res. 52, 416–423.

Brady, T.G., O'Donovan, C.I., 1965. A study of the tissue distribution of adenosine deaminase in six mammal species. Comp. Biochem. Physiol. 14, 101–120.

Chan, T.S., 1979. Purine excretion by mouse peritoneal macrophages lacking adenosine deaminase activity. Proc. Natl. Acad. Sci. USA 76, 925–929.

Chang, Z.Y., Nygaard, P., Chinault, A.C., Kellems, R.E., 1991. Deduced amino acid sequence of *Escherichia coli* adenosine deaminase reveals evolutionarily conserved amino acid residues: implications for catalytic function. Biochemistry 30, 2273–2280.

Charlab, R., Rowton, E.D., Ribeiro, J.M., 2000. The salivary adenosine deaminase from the sand fly Lutzomyia longipalpis. Exp. Parasitol. 95, 45–53.

Charlab, R., Valenzuela, J.G., Andersen, J., Ribeiro, J.M., 2001. The invertebrate growth factor/CECR1 subfamily of adenosine deaminase proteins. Gene 267, 13–22.

Chechik, B.E., Baumal, R., Sengupta, S., 1983. Localization and identity of adenosine deaminase-positive cells in tissues of the young rat and calf. Histochem. J. 15, 373–387.

Conway, E.J., Cooke, R., 1939. The deaminases of adenosine and adenylic acid in blood and tissues. Biochem. J. 33, 479–492.

Daddona, P.E., Kelley, W.N., 1981. Characteristics of an aminohydrolase distinct from adenosine deaminase in cultured human lymphoblasts. Biochem. Biophys. Acta 14, 280–290.

Daddona, P.E., Shewach, D.S., Kelley, W.N., Argos, P., Markham, A.F., Orkin, S.H., 1984. Human adenosine deaminase cDNA a complete primary amino acid sequence. J. Biol. Chem. 259, 12101–12106.

Dolezal, T., Gazi, M., Zurovec, M., Bryant, P.J., 2003. Genetic analysis of the ADGF multigene family by homologous recombination and gene conversion in *Drosophila*. Genetics 165, 653–666.

Dutton, F.L., Chovnick, A., 1990. The PurR mutation of *Drosophila melanogaster* confers resistance to purine and 2,6-diaminopurine by elevating adenosine deaminase activity. Mol. Gen. Genet. 220, 172–176.

Ethier, M.F., Chander, V., Dobson Jr., J.G., 1993. Adenosine stimulates proliferation of human endothelial cells in culture. Amer. J. Physiol. 265, H131–H138.

Gebicke-Haerter, P.J., Christoffel, F., Timmer, J., Northoff, H., Berger, M., Van Calker, D., 1996. Both adenosine A1- and A2-receptors are required to stimulate microglial proliferation. Neurochem. Int. 29, 37–42.

Giblett, E.R., 1985. ADA and PNP deficiencies: how it all began. Ann. NY Acad. Sci. 451, 1–8.

Giblett, E.R., Anderson, J.E., Cohen, F., Pollara, B., Meuwissen, H.J., 1972. Adenosine deaminase deficiency in two patients with severely impaired cellular immunity. Lancet 2, 1067–1069.

Hasko, G., Kuhel, D.G., Chen, J.F., Schwarzschild, M.A., Deitch, E.A., Mabley, J.G., Marton, A., Szabo, C., 2000. Adenosine inhibits IL-12 and TNF-[alpha] production via adenosine A2a receptor-dependent and independent mechanisms. FASEB J. 14, 2065–2074.

Heming, B.S., 2003. In: Insect Development and Evolution. Cornell University Press,

Henderson, J.F., Scott, F.W., 1980. Inhibition of animal and invertebrate cell growth by naturally occurring purine bases and ribonucleosides. Pharmacol. Ther. 8, 539–571.

Henderson, J.F., Scott, F.W., Lowe, J.K., 1980. Toxicity of naturally occurring purine deoxyribonucleosides. Pharmacol. Ther. 8, 573–604.

Hershfield, M., Mitchell, B.S., 2001. Immunodeficiency diseases caused by adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency. In: Scriver, C.L., Sly, W.S., Childs, B., Beaudet, D., Valle, D., Kinzler, K.W., Vogelstein, B. (Eds.), The Metabolic and Molecular Bases of Inherited Disease, vol. 2, Eighth ed. McGraw–Hill, New York, pp. 2585–2625.

Hirschhorn, R., 1999. Immunodeficiency disease due to deficiency of adenosine deaminase. In: Ochs, H.D., Smith, C.I.E., Puck, J.M. (Eds.), Primary Immunodeficiency Diseases. Oxford University Press, New York, pp. 121–139.

Hodge, L.D., Glassman, E., 1967. Purine catabolism in *Drosophila melanogaster*. II. Guanine deaminase, inosine phosphorylase and adenosine deaminase activities in mutants with altered xanthine dehydrogenase activities. Genetics 57, 571–577.

Homma, K., Matsushita, T., Natori, S., 1996. Purification, characterization, and cDNA cloning of a novel growth factor from the conditioned medium of NIH-Sape-4, an embryonic cell line of *Sarcophaga peregrina* (flesh fly). J. Biol. Chem. 271, 13770–13775.

Homma, K.J., Tanaka, Y., Matsushita, T., Yokoyama, K., Matsui, H., Natori, S., 2001. Adenosine deaminase activity of insect-derived growth factor is essential for its growth factor activity. J. Biol. Chem. 276, 43761–43766.

Kawamura, K., Shibata, T., Saget, O., Peel, D., Bryant, P.J., 1999. A new family of growth factors produced by the fat body corre and active on *Drosophila* imaginal disc cells. Development 126, 211–219.

Kuttesch, J.F., Schmalstieg, F.C., Nelson, J.A., 1978. Analysis of adenosine and other adenine compounds in patients with immunodeficiency diseases. J. Liq. Chromatogr. 1, 97–109.

Li, S., Aksoy, S., 2000. A family of genes with growth factor and adenosine deaminase similarity are preferentially expressed in the salivary glands of *Glossina m. morsitans*. Gene 252, 83–93.

MacLaughlin, M., Martinez-Salgado, C., Eleno, N., Olivera, A., Lopez-Novoa, J.M., 1997. Adenosine activates mesangial cell proliferation. Cell. Signaling 9, 59–63.

Maier, S.A., Podemski, L., Graham, S.W., McDermid, H.E., Locke, J., 2001. Characterization of the adenosine deaminase-related growth factor (ADGF) gene family in *Drosophila*. Gene 280, 27–36.

Matsushita, T., Fujii-Taira, I., Tanaka, Y., Homma, K.J., Natori, S., 2000. Male-specific IDGF, a novel gene encoding a membrane-bound extracellular signaling molecule expressed exclusively in testis of *Drosophila melanogaster*. J. Biol. Chem. 275, 36934–36941.

Meininger, C.J., Schelling, M.E., Granger, H.J., 1988. Adenosine and hypoxia stimulate proliferation and migration of endothelial cells. Am. J. Physiol. 255, H554–H562.

Merighi, S., Mirandola, P., Milani, D., Varani, K., Gessi, S., Klotz, K.N., Leung, E., Baraldi, P.G., Borea, P.A., 2002. Adenosine receptors as mediators of both cell proliferation and cell death of cultured human melanoma cells. J. Invest. Dermatol. 119, 923–933.

Migchielsen, A.A., Breuer, M.L., Hershfield, M.S., Valerio, D., 1996. Full genetic rescue of adenosine deaminase-deficient mice through introduction of the human gene. Hum. Mol. Genet. 5, 1523–1532.

Migchielsen, A.A.J., Breuer, M.L., van Roon, M.A., te Riele, H., Zurcher, C., Ossendorp, F., Toutain, S., Hershfield, M.S., Berns, A., Valerio, D., 1995. Adenosine deaminase-deficient mice die perinatally, exhibiting liver-cell degeneration, atelectasis and small intestinal death. Nature Genet. 10, 279–287.

Mohandas, T., Sparkes, R.S., Passage, M.B., Sparkes, M.C., Miles, J.H., Kaback, M.M., 1980. Regional mapping of ADA and ITP chromosome 20: cytogenetic and somatic cell studies in an X/20 translocation. Cytogenet. Cell Genet. 26, 28–35.

Mohandas, T., Sparkes, R.S., Suh, E.J., Hershfield, M.S., 1984. Regional localization of the human genes for adenosylhomocysteine hydrolase (cen——q131) and adenosine deaminase (q131——qter) on chromosome 20. Hum. Genet. 66, 292–295.

Peyot, M.L., Gadeau, A.P., Dandre, F., Belloc, I., Dupuch, F., Desgranges, C., 2000. Extracellular adenosine induces apoptosis of human arterial smooth muscle cells via A(2b)-purinoceptor. Circ. Res. 86, 76–85.

Riazi, M.A., Brinkman-Mills, P., Nguyen, T., Pan, H., Phan, S., Ying, F., Roe, B.A., Tochigi, J., Shimizu, Y., Minoshima, S., Shimizu, N., Buchwald, M., McDermid, H.E., 2000. The human homolog of insect-derived growth factor, CECR1, is a candidate gene for features of cat eye syndrome. Genomics 64, 277–285.

Ribeiro, J.M.C., Charlab, R., Valenzuela, J.G., 2001. The salivary adenosine deaminase activity of the mosquitoes *Culex quinquefasciatus* and *Aedes aegypti*. J. Exp. Biol. 204, 2001–2010.

Schrier, S.M., van Tilburg, E.W., van der Meulen, H., Ijzerman, A.P., Mulder, G.J., Nagelkerke, J.F., 2001. Extracellular adenosine-induced apoptosis in mouse neuroblastoma cells studies on involvement and adenosine receptors and adenosine. Biochem. Pharmacol. 61, 417–425.

Sherwood, A., Stollar, V., 1982. Aedes albopictus cells resistant to adenosine because of a defect in nucleoside transport. Somatic Cell Genet. 8, 575–585.

Sossin, W., Kreiner, T., Barinaga, M., Schilling, J., Scheller, R., 1989. A dense core vesicle protein is restricted to the cortex of granules in the exocrine atrial gland of *Aplysia californica*. J. Biol. Chem. 264, 16933–16940.

Swofford, D.L., 1998. PAUP*4.0—Phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, MA.

Tischfield, J.A., Creagan, R.P., Nichols, E.A., Ruddle, F.H., 1974. Proceedings: assignment of a gene for adenosine deaminase to human chromosome 20. Hum. Heredity 24, 1–11.

Valerio, D., Duyvesteyn, M.G.C., van Ormondt, H., Meera Khan, P., van der Eb, A.J., 1984. Adenosine deaminase (ADA) deficiency in cells derived from humans with severe combined immunodeficiency disease is due to aberration of the ADA protein. Nucleic Acids Res. 12, 1015–1024.

Wakamiya, M., Blackburn, M.R., Jurecic, R., McArthur, M.J., Geske, R.S., Cartwright Jr., J., Mitani, K., Vashnav, S., Belmont, J.W., Kellems, R.E., Finegold, M.J., Montgomery Jr., C.A., Bradley, A., Caskey, C.T., 1995. Disruption of the adenosine deaminase gene causes hepatocellular impairment and perinatal lethality in mice. Proc. Natl. Acad. Sci. USA 92, 3673–3677.

Wiginton, D.A., Adrian, G.S., Hutton, J.J., 1984. Sequence of human adenosine deaminase cDNA including the coding region and a small intron. Nucleic Acids Res. 12, 2439–2446.

Wilson, D.K., Rudolph, F.B., Quiocho, F.A., 1991. Atomic structure of adenosine deaminase complexed with a transition-state analog: understanding catalysis and immunodeficiency mutations. Science 252, 1278–1284.

Wintrobe, M.M., 1959. Clinical Hematology, fourth ed. Lea and Febiger, Philadelphia.

Witte, D.P., Wiginton, D.A., Hutton, J.J., Aronow, B.J., 1991. Coordinate developmental regulation of purine catabolic enzyme expression in gastrointestinal and postimplantation reproductive tracts. J. Cell Biol. 115, 179–190.

Zurovec, M., Dolezal, T., Gazi, M., Pavlova, E., Bryant, P.J., 2001. ADGFs- growth factors with enzymatic activity. Annual Drosophila Research Conference, vol. 42, p. 69.

Zurovec, M., Dolezal, T., Gazi, M., Pavlova, E., Bryant, P.J., 2002. Adenosine deaminaserelated growth factors stimulate cell proliferation in *Drosophila* by depleting extracellular adenosine. Proc. Natl. Acad. Sci. USA 99, 4403–4408.