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7. *lin-4(e912); mut-6(st702); vels13* progeny were screened for Tc1-induced *lin-4* suppressors. *vels13* is an integrated array containing a *col-19::gfp* fusion (*8*), which is expressed adult-specifically in wild-type animals, and because its expression depends on hypodermal terminal differentiation, it is not expressed in *lin-4* mutants. We isolated 34 mutants with restored *col-19::gfp* expression from roughly 10^6 animals screened. Two putative *lin-42* mutations were outcrossed to wild-type males, yielding *lin-42(ve27)* and *ve32*. Several independent strains of *ve27* and *ve32* were outcrossed seven times, and a polymorphic 6.2-kb Eco RI restriction fragment that contained the transposon Tc1 and cosegregated with the *lin-42* mutant phenotype was identified and cloned. When sequences flanking the Tc1 insertion site in the 6.2-kb fragment were used in Southern blot analysis, they detected a polymorphism in genomic DNA from *ve27* and *ve32*, but not from wild-type (N2) animals, the parental strain, or several *lin-42* mutants induced by other means.
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10. A cDNA pool was prepared with an oligo$(dT)_{20}$ primer as described (*34*), and *lin-42* cDNA was amplified with primers from exons 2 and 5. An aliquot was reamplified with the exon 2 primer and a nested exon 5 primer. The DNA sequence of the product matched the GeneFinder prediction. Additional analyses confirmed the exon 1–2 splice pattern and revealed the trans-spliced leader SL1 at the 5′ end and no alternatively spliced products.
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17. Developmentally staged cDNA pools (*16*) were PCR-amplified with primers specific for *lin-42*, *tim-1*, or *ama-1*. The primer sets were chosen to span an intron-exon boundary to distinguish cDNA- and genomic DNA–derived products. *ama-1* and *lin-42* reactions were incubated at 94°C for 1 min, followed by 27 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 20 s with a PTC-100 thermocycler (MJ Research, Waltham, MA). For *tim-1* reactions, the extension time was 45 s. Reactions were terminated before any reagent became limiting. Dilutions of input cDNA and variation of amplification cycle numbers both indicated that the reactions were linear under these conditions. The amplified products were analyzed by Southern (DNA) blot using an end-labeled internal oligonucleotide as a probe (*34*). The blots were analyzed with a PhosphoImager and Image Quant software (Molecular Dynamics). The relative abundance of test cDNA (*lin-42* or *tim-1*) is expressed as the ratio of the test signal to that of *ama-1*. The amplifications and Southern blots were repeated three or four times for each gene. In each experiment, the time point with the highest value was set equal to one, and the remaining samples were expressed as a fraction thereof. The numbers for each time point were then averaged for each gene, and the ratios of *lin-42* and *tim-1* signals relative to *ama-1* were graphed (Fig. 3). The data do not measure the absolute levels of *lin-42* or *tim-1*. Complementary DNA pools for animals reared at 20°C were made as described (*16*).
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35. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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An Adenosine Deaminase that Generates Inosine at the Wobble Position of tRNAs

André P. Gerber and Walter Keller*

Several transfer RNAs (tRNAs) contain inosine (I) at the first position of their anticodon (position 34); this modification is thought to enlarge the codon recognition capacity during protein synthesis. The tRNA-specific adenosine deaminase of *Saccharomyces cerevisiae* that forms I_{34} in tRNAs is described. The heterodimeric enzyme consists of two sequence-related subunits (Tad2p/ADAT2 and Tad3p/ADAT3), both of which contain cytidine deaminase (CDA) motifs. Each subunit is encoded by an essential gene (*TAD2* and *TAD3*), indicating that I_{34} is an indispensable base modification in elongating tRNAs. These results provide an evolutionary link between the CDA superfamily and RNA-dependent adenosine deaminases (ADARs/ADATs).

It has been known for 35 years that inosine occurs at the wobble position of tRNA anticodons (*1*, *2*), and it was postulated that these tRNAs can translate three codons ending in U, C, or A (*3*). This important modification occurs in eight cytoplasmic tRNAs in higher eukaryotes (seven in yeast) and in $tRNA_2^{Arg}$ from prokaryotes and plant chloroplasts (*4*). I_{34} is thereby formed by hydrolytic deamination of a genomically encoded adenosine (A), and the enzymatic activity for this RNA editing reaction has been partially purified from yeast (*5*, *6*). A family of mammalian adenosine deaminases (ADARs) that convert A to I in double-stranded RNA (dsRNA) and edit diverse cellular mRNA precursors (pre- mRNAs) has been identified (*7*), but none of these enzymes forms I in tRNA. Recently, a yeast and human protein that acts on tRNA has been cloned by sequence

Department of Cell Biology, Biozentrum, University of Basel, 4056 Basel, Switzerland.

*To whom correspondence should be addressed. E-mail: walter.keller@unibas.ch

homology to the deaminase domain of the ADAR proteins. This protein, termed Tad1p/ADAT1, specifically deaminates A at position 37 (3′ of the anticodon) in eukaryotic $tRNA^{Ala}$ (*8*, *9*). The yeast *TAD1* gene is not essential for cell viability, and the function of I_{37} in $tRNA_1^{Ala}$, which is further methylated to N^1-methylinosine, is unknown (*8*).

A search of the *Saccharomyces cerevisiae* genome for open reading frames (ORFs) encoding putative deaminases revealed a hypothetical ORF (YJL035c/YJD5) that contains a PROSITE pattern (accession number PS00903) (*10*) characteristic of cytidine/deoxycytidylate deaminases (CDAs). To investigate the physiological relevance of YJL035c, we deleted one of the two copies of the gene in a diploid yeast strain (*11*). Sporulation and tetrad analysis of heterozygotes showed that these *tad2*Δ segregants were not viable.

To confirm that *TAD2* is required for vegetative growth, we transformed *tad2*Δ heterozygotes with pFL38-Tad2 (a centromeric plasmid containing both *TAD2* and *URA3*) or pGAL-$FLIS_6$-Tad2 (a centromeric plasmid with *URA3* bearing the coding sequence of *TAD2* fused to a 5′-FLAG and a 3′-hexahistidine epitope) (*12*). In both cases dissection of transformants resulted in four viable spores, and *tad2*Δ segregants containing pFL38-Tad2 or pGAL-$FLIS_6$-Tad2, respectively, were isolated. When these cells were grown on 5-fluoro-orotic acid (5-FOA), which is toxic to cells expressing *URA3*, no colonies appeared at either 23° or 30°C, showing that *TAD2* is an essential gene.

We next isolated a temperature-sensitive (ts) *tad2* allele to functionally characterize *TAD2* (*13*). Cells bearing the *tad2-1* allele grew normally at 23°C but could not form colonies at 37°C. A cell-free extract prepared from these cells lacked specific tRNA:A34 deaminase activity as measured by incubation with synthetic [^{33}P]ATP (adenosine 5′-triphosphate)–labeled yeast $tRNA_1^{Ala}$ (*5*, *8*), which is a natural substrate (*1*, *2*). Activity was restored by addition of purified recombinant Tad2 protein (rTad2p) to the extract (*14*). Furthermore, $tRNA^{Ala}$ isolated from *tad2-1* mutant cells contained unmodifed A_{34}, indicating that Tad2p is involved in deamination in vivo (*14*). However, rTad2p alone had no deaminase activity. These results suggested that Tad2p is necessary but not sufficient to form I_{34} in tRNAs and that an additional component is required.

To isolate this factor, we cultured *tad2*Δ [pGal-$FLIS_6$-Tad2] cells and purified the Tad2 fusion protein (*15*). A protein of 38 kD copurified in a 1:1 ratio with the tagged Tad2p (Fig. 1, A and B), and fractions containing both Tad2p and p38 converted A_{34} to I_{34} in synthetic $tRNA^{Ala}$ (Fig. 1C). To identify p38, the masses of lysine C–digested peptides were determined by mass spectrometry. Nine protein fragments matched the putative *S. cerevisiae* ORF YLR136c [Munich Information Center for Protein Sequences (MIPS) accession number S53395]. Polymerase chain reaction (PCR) and sequence analysis on yeast cDNA with primers annealing upstream of YLR136c and at the stop codon confirmed the presence of two introns (*16*). Thus, this gene, named *TAD3*, represents the fifth gene in *S. cerevisiae* that contains two introns (*17*).

We tested the functionality of these cDNAs in vivo by disrupting one allele of *TAD3* (*11*). Tetrad dissection of the heterozygotes showed that *tad3*Δ segregants were not viable. *tad3*Δ segregants containing the plasmid pFL38-Tad3 (*CEN-URA3*) (*16*) could be isolated. After plating the cells on 5-FOA, the cells grew only when *TAD3* was provided on a second plasmid with *LEU2* (pFL36-Tad3) or *ADE2* (pGal-FL-Tad3) markers (*18*). In contrast, *tad3*Δ cells that expressed Tad3p from the intronic ATG_2 (ΔNTad3p) had a slight growth defect at 23°C and were nonviable at 37°C (*18*). Extracts from these ts cells (*tad3-1*) lacked tRNA:A34 editing activity, indicating that Tad3p is essential for A_{34} deamination (*14*). The requirement of *TAD2* and *TAD3* for cell viability strongly suggests that inosine at the wobble position of tRNAs is an essential modification in yeast.

To confirm that Tad2p and Tad3p com-

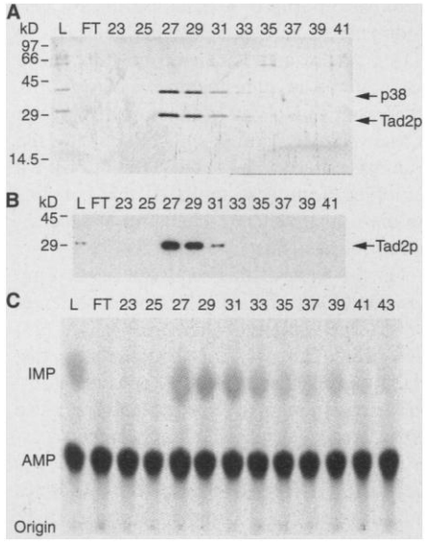

Fig. 1. Purification of the tRNA A:34 deaminase. **(A)** Samples (5 μl) of the final MonoQ column fractions were subjected to electrophoresis on a 12% SDS-polyacrylamide gel, and proteins were stained with silver. Load (L) and fraction numbers are indicated at the top, and molecular masses on the left (in kilodaltons). **(B)** Immunoblot analysis with a mouse anti-FLAG M2 monoclonal antibody (1:5000 dilution). **(C)** tRNA-specific adenosine deaminase assay (*8*). [α-^{33}P]ATP-labeled yeast $tRNA^{Ala}$ (200 fmol) was incubated with samples (0.2 μl) of column fractions for 45 min at 30°C. After ethanol precipitation the RNA was digested with P1 nuclease and reaction products were separated by one-dimensional thin-layer chromatography. The chromatographic origin and the migration positions of adenosine monophosphate (AMP) and inosine monophosphate (IMP) are indicated on the left.

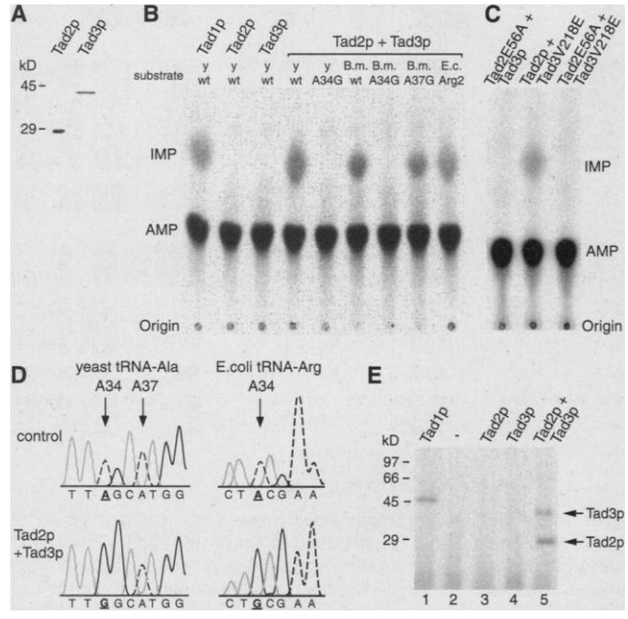

Fig. 2. Reconstitution of the tRNA A:34 deaminase with recombinant Tad2p and Tad3p. **(A)** Purified rTad2p and rTad3p were separated by SDS-PAGE and stained with Coomassie blue. **(B)** Recombinant proteins (10 ng) were used in tRNA-specific adenosine deaminase assays (*8*). Abbreviations: y, yeast; B.m., *B. mori*; E.c., *E. coli*; A34G, mutant tRNA containing G_{34} instead of A_{34}; A37G, G_{37} instead of A_{37}. **(C)** Mutant rTad2 and rTad3 proteins were assayed on WT $tRNA^{Ala}$ of *B. mori*. **(D)** Sequence analysis of edited yeast and *E. coli* tRNAs. After incubation of synthetic tRNAs with rTad2p-rTad3p or buffer (control), tRNAs were reverse transcribed and amplified by PCR and products were sequenced. Only the anticodon loop-region is shown. Nucleotide position 34 is underlined, and A peaks are dashed. Because inosine base-pairs with C, I is represented as G. **(E)** UV cross-linking of Tad1p and rTad2p-rTad3p to labeled WT yeast $tRNA^{Ala}$. Reactions (10 μl) were carried out with 200 ng of each recombinant protein and 250 fmol of labeled $tRNA^{Ala}$ in assay buffer. After incubation for 15 min at room temperature, the reactions were irradiated on ice in a UV Stratalinker at 400 mJ and digested with 250 ng of ribonuclease A for 30 min at 37°C. Proteins were separated on denaturing SDS–12% polyacrylamide gels and exposed on a PhosphorImager screen (Molecular Dynamics).

pose the tRNA:A34 deaminase, we reconstituted the activity with purified recombinant proteins (Fig. 2) (*19*). The combination of rTad2p and rTad3p, but neither protein alone, specifically converted A_{34} to I_{34} in tRNAs from yeast and the silkworm *Bombyx mori* (Fig. 2, B and D). In addition, the in vitro–reconstituted deaminase was active on *Escherichia coli* $tRNA_2^{Arg}$, yeast $tRNA_2^{Ser}$ (another natural substrate), and a mutant yeast $tRNA^{Asp}$ in which the anticodon loop was exchanged with that of yeast $tRNA_2^{Arg}$ (*5*). In contrast, removal of one base from the 7-nucleotide anticodon loop in yeast $tRNA^{Ala}$ (ΔU33) abolished deaminase activity (*14*). A mixture of Tad2p and Tad3p, but neither protein alone, could be ultraviolet (UV) cross-linked to yeast $tRNA^{Ala}$ (Fig. 2E) or *B. mori* $tRNA^{Ala}$ (*14*). This may indicate that association of the two subunits is required for tRNA binding. To further analyze the Tad2p-Tad3p complex, we combined the recombinant proteins and ran them on a sizing column. rTad2p-rTad3p coeluted at an estimated molecular mass of 70 kD together with tRNA:A34 deaminase activity (*2*), indicating that Tad2p and Tad3p form a heterodimeric complex. This type of subunit composition has not been observed in other deaminases. CDAs form homodimeric or homotetrameric protein complexes (*20*), whereas ADARs and Tad1p/ADAT1 act as monomers (*7*, *8*).

The COOH-terminal part of Tad3p is related in sequence to Tad2p (26% amino acid identity and 45% similarity in 120–amino acid overlap) (*2*). The homologous region contains the deaminase motif signatures found in the CDA superfamily including the catalytic subunit APOBEC-1, which converts C to U in mammalian apolipoprotein B pre-mRNA (*20*, *21*) (Fig. 3). In particular, Tad2p-Tad3p share the three key amino acids (His, Cys, Cys) involved in zinc coordination and the proline in the deaminase motif II that acts as the ammonium group binding site. Furthermore, Tad2p contains a conserved glutamate (Glu^{56}) that is required for proton shuttling during catalysis in CDAs (*20*). A Tad2p point mutant, where Glu^{56} was substituted by Ala (E56A) (*22*), combined with wild-type (WT) Tad3p, had no deaminase activity in vitro (Fig. 2C). In Tad3p, this Glu is replaced by valine (Val^{218}) (framed in Fig. 3B). The Tad3 point mutant V218E acted like the WT protein and could not substitute for the E56A mutation in Tad2p (Fig. 2C). Therefore, Tad2p most likely represents the catalytic subunit of the tRNA:A34 deaminase.

Database searches identified a number of sequences related to Tad2p and Tad3p, suggesting that these putative deaminases are orthologs (Fig. 3) (*2*). Remarkably, the prokaryotic genomes encoded only one such homologous polypeptide. Therefore, Tad2p and Tad3p may be paralogs that appeared after the divergence of prokaryotes and eukaryotes by genome duplication. Through this event and further genetic drift, the eukaryotic tRNA:A34 deaminases could have acquired the ability to modify additional tRNA substrates. This is consistent with the fact that the *E. coli* tRNA:A34 deaminase cannot modify any of the seven yeast tRNAs containing I_{34}, whereas the yeast enzyme can modify *E. coli* $tRNA_2^{Arg}$ (*5*).

The Tad2-Tad3 protein family appears to be positioned evolutionarily between the proteins of the CDA superfamily and the members of the ADAR family including Tad1p/ADAT1. Tad2p-Tad3p share the indicative deaminase motifs of CDAs and form a heterodimeric complex. But in contrast to CDAs, Tad2p and Tad3p do not deaminate free cytidine or cytosine in vitro (*23*, *14*). Moreover, Tad2p-Tad3p functionally belong to the ADAR family. ADARs share the deaminase motif I and II of CDAs but have a conserved deaminase motif III that contains the third putative Zn^{2+}-chelating residue required for catalysis (*24*) (Fig. 3).

On the basis of this sequence relationship between CDAs and ADARs and considering the structural differences between CDA (*21*) and ADA (*25*), it was hypothesized that ADARs evolved from a CDA precursor (*26*). Our results substantiate this hypothesis and we further propose that Tad2p-Tad3p is the ancestor of the deaminase domain found in ADARs and Tad1p. After the divergence of prokaryotes and eukaryotes, a Tad2p-like enzyme might have further evolved by establishment of deaminase motif III and changing site specificity from position 34 to position 37 in one tRNA substrate. This Tad1p-like protein might then have evolved to the metazoan ADARs by the acquisition of dsRNA-binding modules (*8*).

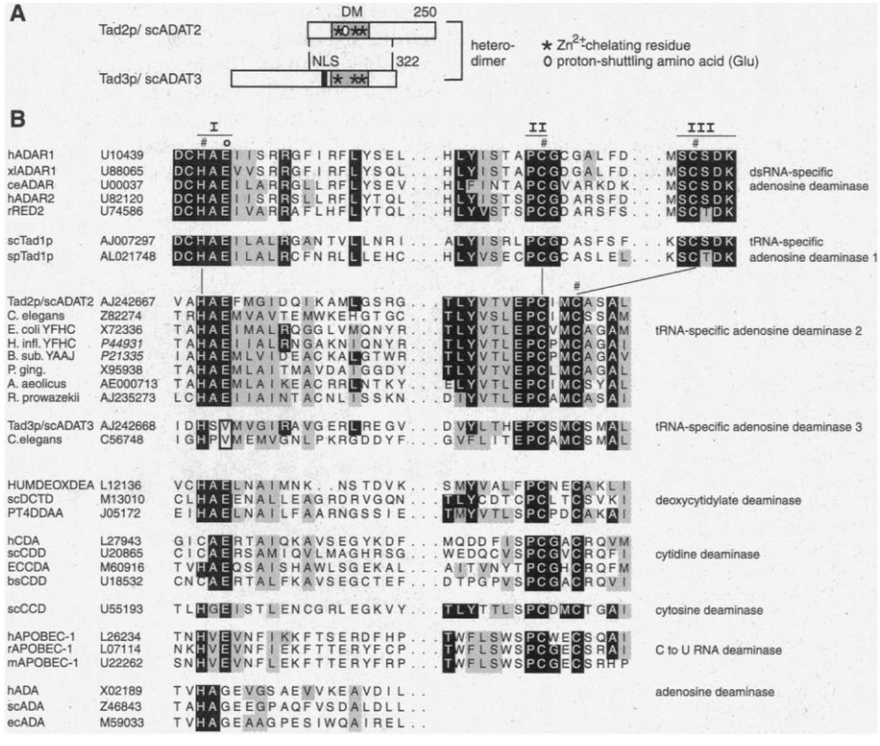

Fig. 3. Tad2p and Tad3p contain a deaminase domain that is similar to those of CDAs and ADARs/ADAT1. (**A**) Protein domain organization of Tad2p and Tad3p. The deaminase domain (DM) is boxed in gray. A putative nuclear localization signal (NLS) in Tad3p (black box), the region of sequence similarity between Tad2p and Tad3p (dashed line), and the lengths of the proteins (in amino acids) are indicated. (**B**) Multiple sequence alignment of deaminase domains. Highly conserved residues found in at least two different enzymes are framed in black; similar ones are in gray. The deaminase motifs I, II, and III are overlined, and the putative Zn^{2+}-chelating residues (#) and proton-transferring amino acid (o) are indicated. The DNA Data Bank of Japan/European Molecular Biology Laboratory/GenBank accession numbers are indicated in the column next to the names of the enzymes. Abbreviations: *C. elegans*, *Caenorhabditis elegans*; *E. coli*, *Escherichia coli*; *H. infl.*, *Haemophilus influenzae*; *B. sub.*, *Bacillus subtilis*; *P. ging.*, *Porphyromonas gingivalis*; *A. aeolicus*, *Aquifax aeolicus*; and *R. prowazekii*, *Rickettsia prowazekii*. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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12. The primers YJR1 (5′-CGCGGATCCTTGAGACTACTCTTGGGGAC-3′) annealing 300 base pairs (bp) upstream and YJR2 (5′-ATCGAATTCTACGAAAGGATAC-3′) annealing 209 bp downstream of the ORF YJL035c were used to amplify *TAD2* from genomic DNA by PCR and cloned into pCR2.1 (Invitrogen). The Eco RI fragment containing *TAD2* was subcloned into plasmid pFL38 (*27*), resulting in pFL38-Tad2. The ORF of YJL035c was amplified with primers YJ1 (5′-GGACTAGTGCAGCATATTAAACATATGAGG-3′) and YJ2 (5′-CGACTAGTTTAGATTTCTTATGTACATTAAAC-3′) and cloned by means of Spe I (underlined) into pGalΔTrp-FLIS$_6$-Tad1 (*7*) to generate pGal-FLIS$_6$-Tad2.
13. A library of mutant *TAD2* alleles was made by PCR-mediated mutagenesis and transformed into *tad2*Δ::*TRP1*[pFL38-Tad2] yeast cells. Replica plating on FOA and selection of ts strains resulted in the isolation of the *tad2-1* allele in which codons for Cys^{126} (TGT) and Leu^{153} (CTG) are changed to Ser (AGT) and Pro (CCG), respectively.
14. A. P. Gerber and W. Keller, data not shown.
15. Cells were cultured in 10 liters of rich medium containing 2% galactose. The cell pellet (165 g) was washed with buffer A [50 mM tris-HCl (pH 7.9), 100 mM KCl, 10% glycerol, 1 mM β-mercaptoethanol, 0.01% NP-40, 0.5 mM phenylmethylsulfonyl fluoride, pepstatin (0.7 μg/ml), and leupeptin (0.4 μg/ml)], and extracts were prepared in liquid nitrogen. After centrifugation, the cleared supernatant was passed over Ni^{2+}-nitrilotriacetic acid (NTA) agarose (Qiagen), and bound proteins were eluted with 250 mM imidazole. Fractions containing FLAG-Tad2p-His$_6$ were passed twice over 400 μl of an anti–M2-FLAG affinity matrix (Kodak) and eluted with buffer B [buffer A containing 25 mM KCl, 0.1 mM EDTA, and 0.1 mM dithiothreitol (DTT) instead of β-mercaptoethanol] supplemented with FLAG-peptide (50 μg/ml) (Kodak). Fractions containing tagged Tad2p were loaded on a MonoQ column (PC 1.6/5; Smart System, Pharmacia) that had been equilibrated in buffer C (buffer B containing 0.2 mM EDTA and 1 mM DTT) and developed with a 2-ml gradient from 25 to 300 mM KCl. Fifty-microliter fractions were collected. Fractions with tRNA editing activity eluted at 145 mM KCl (~4 μg).
16. The comparison of theoretical with experimentally determined peptide masses indicated that the putative ATG in S53395 (ATG$_2$) is not the correct start codon but part of a second intron. To confirm this, we amplified the *TAD3* ORF with primers YL8 (5′-GGACTAGTTAAGAAAGTTAATAATCCGC-3′) and YL2 (5′-CGACTAGTCCGCAGCAGACATCCCGGTCAAC-3′) on *S. cerevisiae* cDNA. Spe I sites for subcloning are underlined. *TAD3* with introns was obtained by PCR on genomic DNA with primers YLreg1 (5′-GGTCTGTAGATCAATGTCAAGC-3′) annealing 237 bp upstream and YLreg2 (5′-GTTCAAGCAGCAACTACAGTCG-3′) hybridizing 386 bp downstream of the *TAD3* ORF. The fragment was further cloned into pFL38 and pFL36, respectively (*27*).
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18. In pGal-FL-Tad3, the ORF of *TAD3* with a NH$_2$-terminal FLAG epitope is on a plasmid containing *ADE2* and the Gal promoter. pGal-FL-ΔNTad3 was generated with primer YL1 (5′-GGACTAGTGAGATCTAACAGAAATCAGGATC-3′) annealing at ATG$_2$ (*15*).
19. A Nco I–Eco RI fragment from pGal-FLIS$_6$-Tad2 coding for the ORF of *TAD2* with an NH$_2$-terminal FLAG epitope and a COOH-terminal 6xHis-tag was subcloned into pTrcHisB (Invitrogen), resulting in pTrc-FLIS$_6$-Tad2. The ORF of *TAD3* (*16*) was subcloned by means of Spe I, generating pTrc-FLIS$_6$-Tad3. Plasmids were transformed into BL21 (DE3). *Escherichia coli* cells and protein expression were induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside for 5 hours at 23°C. Cells were lysed by sonication and the extract was fractionated on a Ni-NTA column and on anti–FLAG-M2 affinity matrix. rTad3p was further purified on a 1-ml HiTrap Q column (Pharmacia).
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Regulation of Lineage Commitment Distinct from Positive Selection

Robert Keefe, Vibhuti Dave, David Allman, David Wiest, Dietmar J. Kappes*

Developing αβ T cells diverge into the CD4 and CD8 lineages as they mature in the thymus. It is unclear whether lineage commitment is mechanistically distinct from the process that selects for the survival of T cells with useful T cell receptor (TCR) specificities (positive selection). In HD mice, which lack mature CD4$^+$ T cells, major histocompatibility complex (MHC) class II–restricted T cells are redirected to the CD8 lineage independent of MHC class I expression. However, neither TCR-mediated signaling nor positive selection is impaired. Thus, the HD mutation provides genetic evidence that lineage commitment may be mechanistically distinct from positive selection.

Developing αβ thymocytes go through three major phenotypic stages, first expressing neither CD4 nor CD8 (double negative; DN), then expressing both (double positive; DP), and finally expressing only one or the other (CD4$^+$8$^-$ or CD4$^-$8$^+$) (SP). At the DP stage thymocytes are selected to undergo the alternative outcomes of negative selection, positive selection, or death by neglect depending on the interaction of the αβTCR complex with intrathymic major histocompatibility complex ligands (*1, 2*). Coincident with positive selection, thymocytes undergo lineage commitment, a process that ensures the correlation of TCR specificity toward class I or II MHC with the cell's functional phenotype as a CD8$^+$ killer or a CD4$^+$ helper T cell. Various mechanisms have been proposed to explain how this correlation is achieved (*3–6*). At the molecular level CD4 and CD8 coreceptors (*7, 8*) as well as the Ras-MAPK (*9*) and Notch (*10*) pathways are likely to play a role. The mutant HD mouse (*11*) is deficient in generation of peripheral CD4$^+$ T cells because of a specific defect in thymic development not affecting antigen presentation or CD4 function, distinct from other spontaneous and induced mouse mutants with similar phenotypes (*12*). Although the HD defect was shown to be intrinsic to the hematopoietic lineage (*11*), it is unclear whether it maps to thymocytes or to bone marrow–derived nonthymocytes, which could cause aberrant negative selection of class II–restricted thymocytes.

To test this, we cotransferred bone marrow from *HD*$^{-/-}$ and *HD*$^{+/+}$ mice into the same *RAG*$^{-/-}$ recipients (*13*). If another cell type were acting in trans to prevent thymocytes from

Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111, USA.

*To whom correspondence should be addressed. E-mail: dj_kappes@fccc.edu

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