Transgenic mice overexpressing ornithine and S-adenosylmethionine decarboxylases maintain a physiological polyamine homoeostasis in their tissues

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Recent work has shown that transgenic mice overexpressing human ornithine decarboxylase display no marked changes in the tissue concentrations of spermidine or spermine in spite of a dramatic increase in putrescine levels. In the tissues of transgenic mice carrying the human spermidine synthase gene and in those of hybrid mice overexpressing both ornithine decarboxylase and spermidine synthase, spermidine and spermine levels remain within normal limits. To test whether the amount of the propylamine group donor, decarboxylated S-adenosylmethionine, limits the conversion of putrescine into the higher polyamines, we have produced transgenic mouse lines harbouring the rat S-adenosylmethionine decarboxylase gene in their

genome. However, neither these mice nor the hybrid mice overexpressing both ornithine decarboxylase and S-adenosylmethionine decarboxylase displayed significant changes in their spermidine and spermine tissue levels. To study the mechanism by which cells maintain the constancy of the polyamine concentrations, we have determined the metabolic flux of polyamines in transgenic primary fibroblasts using pulse labelling. The results indicate that the polyamine flow is faster in transgenic primary fibroblasts than in non-transgenic fibroblasts and that the intracellular homoeostasis of higher polyamines is maintained at least partly by the acetylation of spermidine and spermine and their secretion into the medium.

INTRODUCTION

Polyamines (putrescine, spermidine and spermine) are essential for mammalian cell growth and differentiation [1–3]. The two key regulatory enzymes in their biosynthesis are ornithine decarboxylase (ODC; 4.1.1.17) and S-adenosylmethionine decarboxylase (AdoMetDC; EC 4.1.1.50). These decarboxylases are very highly regulated *in vivo*, not only at the level of gene expression but also post-transcriptionally by the polyamines themselves (for reviews see [4,5]). This reflects the key role of these enzymes in maintaining the polyamine levels between the physiological limits.

ODC and AdoMetDC possess very short physiological half-lives and are strikingly and transiently induced in cells in culture by a number of growth-promoting agents such as hormones, tumour promoters and growth factors [2,6–9]. Treatment with these agents is associated with an increase in the content of the intracellular polyamines. Such increases are also known to occur in many tissues in response to growth-promoting stimuli, and it has been suggested that the changes in polyamine concentration may play a regulatory role in controlling tissue growth [10,11]. This is in accordance with the findings that the polyamine levels are higher in developing mammalian tissues than in those of mature tissues. The polyamine requirement for cell growth is also supported by studies on cultured cells. The use of specific inhibitors of polyamine-synthesizing enzymes has shown that undisturbed synthesis of polyamines is, in fact, a prerequisite for cell proliferation [9]. To study the regulation of polyamine synthesis and the consequences of overexpression of polyamine-synthesizing enzymes, several transgenic mouse lines carrying multiple copies of the human ODC and/or spermidine

synthase gene have been generated [12–16]. Despite strikingly elevated tissue putrescine concentrations in mice overexpressing the human ODC gene, no or minimal changes were found in the levels of the higher polyamines, spermidine and spermine [13,17]. Overexpression of spermidine synthase also did not significantly affect tissue putrescine, spermidine or spermine levels [16]. Moreover, polyamine levels remained within normal limits in hybrid transgenic mice overexpressing both the human ODC and spermidine synthase genes [16]. These results may indicate that AdoMetDC plays a critical role in the regulation of the tissue polyamine homoeostasis by acting as a limiting step in the conversion of putrescine into the higher polyamines, spermidine and spermine. It is known that the content of decarboxylated S-adenosylmethionine (dcAdoMet) is normally very low in mammalian cells [18,19], and thus the flow of putrescine to the higher polyamines may be regulated by the activity of AdoMetDC and consequently by the amount of its product, dcAdoMet. To test this possibility, we have generated transgenic mouse lines overexpressing rat AdoMetDC gene and cross-breds overexpressing both human ODC and rat AdoMetDC.

MATERIALS AND METHODS

Transgenic animals

A 19.5 kb fragment consisting of the entire rat AdoMetDC gene (AMD1B) [20] flanked by 3 kb of 5' sequences and 0.9 kb of 3' sequences was injected into fertilized mouse oocytes by the standard pronuclear microinjection technique [21]. Fertilized oocytes were obtained from superovulated Balb/cxDBA/2 mice

Abbreviations used: ODC, ornithine decarboxylase; AdoMetDC, S-adenosylmethionine decarboxylase; dcAdoMet, decarboxylated S-adenosylmethionine; RT, reverse transcriptase; AMV, avian myeloblastosis virus.

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mated with males of the same strain. Transgenic mice were identified by PCR analysis of tail DNA samples. Lines were established, and expression of the transgene was analysed in the F_1 or F_2 generations. The offspring of a founder animal, designated UKU99, were used to determine tissue polyamine concentrations and the activities of the enzymes involved in polyamine metabolism. Syngenic littermates served as controls. A hybrid transgenic mouse line was obtained by mating UKU99 mice with transgenic mice overexpressing the human ODC gene (UKU2, previously designated as K2 [12–14]).

Materials

L-[1- 14 C]Ornithine (sp. radioactivity 61 Ci/mol), S-adenosyl-L-[carboxyl- 14 C]methionine (sp. radioactivity 54.6 Ci/mol), [1- 14 C]acetyl-CoA (sp. radioactivity 5 Ci/mol), 5'-[α - 32 P]dCTP (sp. radioactivity 410 Ci/mol) and L-[U- 14 C]ornithine (sp. radioactivity 262 Ci/mol) were purchased from Amersham International (Amersham, Bucks., U.K.). Restriction endonucleases and DNA-modifying enzymes were from Boehringer Mannheim, New England Biolabs, Inc. or Promega Biotec. Avian-myeloblastosis-virus reverse transcriptase (AMV-RT) and RNasin were from Promega. Oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer. Purification of DNA fragments was carried out using Gene Clean II from BIO 101 (La Jolla, CA, U.S.A.) according to the manufacturer's instructions. Reagents for labelling of DNA samples by nick-translation or random priming methods were purchased from Boehringer Mannheim. All chemicals and electrophoresis reagents were from standard sources at the highest grade commercially available. Thermostable DNA polymerase (DynazymeTM) was from Finnzymes Inc. (Espoo, Finland). Other reagents were of molecular-biology grade and purchased from Sigma.

Analytical methods

Polyamine concentrations were determined by Hewlett–Packard HP 1090 liquid chromatography by using the procedure described by Mach et al. [22]. The activities of ODC and AdoMetDC were assayed by the methods of Jänne and Williams-Ashman [23,24].

The rat AdoMetDC-encoding transgene was identified by PCR. For the genomic PCR, mouse chromosomal DNA was isolated as described by Sambrook et al. [25]. The primers, 5'-ACTGGCGCAATGATGCCT-3' and 5'-TCACTAGTCCGCCTTCTG-3', recognized sequences in exon 8 (non-coding region) of the rat gene, but are not present in the mouse counterpart. PCR was carried out using a standard method.

Rat-specific AdoMetDC mRNA was detected with reverse transcriptase (RT)-PCR and subsequent restriction with a *Bgl*II that cuts the amplified fragment derived from the mouse but not that from the rat. Total RNA from mouse tissues was isolated using a guanidinium isothiocyanate extraction [26]. Total RNA (5 μ g) was added to an RT buffer composed of 50 mM Tris, pH 8.3, 40 mM KCl, 7 mM MgCl₂, BSA (0.1 mg/ml), 0.5 mM each dNTP, 1 mM dithiothreitol, 1.25 μ g of upstream primer, 5'-TGCATTAAGAACTC-3' (nucleotides 675–689 of the rat cDNA [27] and nucleotides 659–674 of the mouse cDNA [28] respectively), RNasin (10 units; Promega) and AMV-RT (20 units) in a total volume of 20 μ l. The RT reaction was performed for 1 h at 42 °C. PCR was performed in 100 μ l of solution consisting of 1 $\times$ PCR buffer, 100 μ M dNTPs, 50 pmol of primers [upstream primer as in the RT reaction, downstream primer 5'-AAGGAACCTGAACTT-3' (nucleotides 153–168 of the rat cDNA [27] and nucleotides 137–151 of the mouse cDNA [28]

respectively)] and 5 units of thermostable DNA polymerase. These primers generate a 537 bp fragment from the DNA of both species but only the fragment derived from the mouse DNA contains a *Bgl*II restriction site. Cycling parameters were as follows: denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min. After 25 cycles, the reaction mixture was incubated for 10 min at 72 °C. The PCR product was purified by phenol/chloroform (1:1, v/v) and chloroform extractions. After ethanol precipitation the amplified product was resuspended in 100 μ l of TE buffer (10 mM Tris/1 mM EDTA, pH 8.0). Samples (25 μ l) were digested for 4 h at 37 °C with *Bgl*II.

For the Northern-blot analysis, total RNA was fractionated (15 μ g/lane) on a 1% (w/v) agarose gel containing formaldehyde [29], blotted on to nitrocellulose, and hybridized with a nick-translated cDNA probe (1013 *Pst*I–*Pvu*II fragment of pSAMr1 [30]). The relative changes in mRNA levels were determined from Northern blots using a Molecular Dynamics computing laser densitometer (model 300A), and the data were analysed using the Image Quant program. Density values were standardized to the value for 28 S rRNA hybridization within each experiment.

For the pulse labelling, early-passage mouse embryonic transgenic and non-transgenic fibroblasts were grown in Dulbecco's modified Eagle's medium/20% (v/v) foetal bovine serum at 37 °C. After 16 h (60–80% confluent) the medium was replaced with fresh medium containing 0.4 μ Ci/ml of L-[U- 14 C]ornithine. After 2 h labelling, the medium was removed, the cells were washed three times with PBS and fresh medium was added. The cells were grown for 4–24 h, and the concentrations and radioactivity of the polyamines were determined.

Differences between means were computed using Student's *t* test when applicable. Statistical significance was inferred when $P < 0.05$.

RESULTS AND DISCUSSION

Several different lines of transgenic mice containing the rat AdoMetDC gene were generated by injection of fertilized eggs with a construct containing the native rat AdoMetDC gene. Integration of the rat transgene in founders was established by genomic PCR. In order to evaluate whether the integrated rat AdoMetDC DNA fragment was able to direct the expression of

Figure 1 Demonstration of rat-specific AdoMetDC mRNA in the livers of transgenic mice with RT-PCR

Experimental details are described in the Materials and methods section. For each mouse line: lane 1, non-transgenic; lane 2, non-transgenic digested with *Bgl*II; lane 3, transgenic; lane 4, transgenic digested with *Bgl*II. *Hae*III-digested fragments of ϕ X174 DNA were used as DNA size markers.

Table 1 AdoMetDC activities in the brains and livers of transgenic and non-transgenic mice and increases in the enzyme activity (Fold) in transgenic mice relative to non-transgenic littermates

Results are means of two animals except UKU102 ($n = 1$). The activities are expressed as pmol/h per mg wet wt of tissue. The sex and ages of each group are indicated in parentheses.

Mice	Liver	Fold	Brain	Fold
Control	3.2		17.4	
UKU99 (male, 4–5 weeks)	12.3	3.8	48.5	2.8
Control	2.4		20.9	
UKU101 (male, 4–5 weeks)	4.1	1.7	33.0	1.6
Control	7.7		30.6	
UKU102 (female, 5 weeks)	31.5	4.1	112	3.7
Control	3.2		34.7	
UKU103 (female, 6 weeks)	6.5	2.0	67.1	1.9
Control	3.2		34.7	
UKU104 (male, 6 weeks)	8.2	2.6	104.5	3.0

the rat AdoMetDC gene, total RNA was isolated from the livers of transgenic mice and analysed by RT-PCR. Figure 1 shows the results from four transgenic mouse lines (UKU99, UKU101, UKU103 and UKU104), confirming that the rat transgene is actively transcribed in the liver.

Table 1 summarizes the AdoMetDC activities in the liver and brain tissues of the five different transgenic mice lines (UKU99, UKU101, UKU102, UKU103 and UKU104). The enzyme activity in these tissues varied from approx. 2–4-fold relative to their syngenic littermates. Although increases in the enzyme activity are not dramatic, the magnitude of the changes in the tissue enzyme activities in each transgenic mouse line relative to those of their non-transgenic littermates showed a good correlation, indicating that the expression of the transgene(s) is

Figure 2 Expression of the mouse AdoMetDC gene and the rat transgene in the tissues of transgenic mice (UKU99)

AdoMetDC activity in transgenic animals represents the total tissue activity minus the endogenous mouse activity (the mean enzyme activity in non-transgenic littermates of the same sex). The correlation coefficient of the linear regression is 0.960.

controlled by the tissue-specific control elements. This suggests that the 3-kb-long 5'-flanking region in the transgene construct contains adequate elements for tissue-specific transcription. The genomic Southern-blot analysis from the transgenic mice revealed that the copy number of the transgene is not high in any of the

Table 2 Ornithine and AdoMetDC activities and polyamine concentrations in tissues of transgenic and non-transgenic mice

Results are means $\pm$ S.D. from three animals in a group. Enzyme activities are expressed as pmol/h per mg wet wt. Significance of the differences: ^asignificantly different from the control, $P < 0.001$; ^bsignificantly different from UKU99, $P < 0.001$; ^csignificantly different from the control, $P < 0.01$; ^dsignificantly different from UKU99, $P < 0.01$; ^esignificantly different from UKU2, $P < 0.01$; ^fsignificantly different from the control, $P < 0.05$; ^gsignificantly different from UKU99, $P < 0.02$; ^hsignificantly different from UKU2, $P < 0.02$; ⁱsignificantly different from UKU99, $P < 0.05$; ^jsignificantly different from UKU2, $P < 0.05$; ^ksignificantly different from UKU2, $P < 0.005$. ND, activity below the detection limit of the method.

Tissue	ODC	AdoMetDC	Concentration (nmol/g wet wt)			Spermidine/ spermine
			Putrescine	Spermidine	Spermine	
Testis						
Control	15.2 $\pm$ 8.3	18.4 $\pm$ 0.6	15 $\pm$ 12	783 $\pm$ 64	1063 $\pm$ 51	0.74 $\pm$ 0.03
UKU99	9.1 $\pm$ 1.5	44.4 $\pm$ 4.5 ^c	2 $\pm$ 3	526 $\pm$ 41 ^f	1064 $\pm$ 99	0.50 $\pm$ 0.02 ^c
Brain						
Control	ND	34.7 $\pm$ 1.0	< 1	527 $\pm$ 57	361 $\pm$ 16	1.45 $\pm$ 0.10
UKU99	ND	114.0 $\pm$ 14.7 ^c	< 1	462 $\pm$ 62	397 $\pm$ 26	1.16 $\pm$ 0.17
UKU2	10.3 $\pm$ 0.6	25.9 $\pm$ 3.7 ^b	132 $\pm$ 19	596 $\pm$ 100	374 $\pm$ 42	1.58 $\pm$ 0.17 ⁱ
UKU2/UKU99	7.4 $\pm$ 5.0	92.1 $\pm$ 22.5 ^{c,g}	91 $\pm$ 7 ⁱ	528 $\pm$ 47	342 $\pm$ 44	1.56 $\pm$ 0.30
Spleen						
Control	7.5 $\pm$ 3.0	29.6 $\pm$ 8.6	113 $\pm$ 12	2352 $\pm$ 511	1045 $\pm$ 160	2.29 $\pm$ 0.61
UKU99	6.5 $\pm$ 1.3	49.1 $\pm$ 7.1 ^f	80 $\pm$ 2 ^f	1606 $\pm$ 140	1258 $\pm$ 112	1.27 $\pm$ 0.14 ^f
UKU2	57.0 $\pm$ 4.3 ^{a,b}	24.6 $\pm$ 8.6 ^g	226 $\pm$ 34 ^{c,d}	2433 $\pm$ 718	858 $\pm$ 25 ^j	2.82 $\pm$ 0.80 ^j
UKU2/UKU99	89.0 $\pm$ 33.0 ^{c,d}	56.0 $\pm$ 9.7 ^h	125 $\pm$ 21 ^j	1771 $\pm$ 74	1202 $\pm$ 31 ^k	1.47 $\pm$ 0.08
Heart						
Control	3.7 $\pm$ 0.4	9.3 $\pm$ 3.1	< 1	289 $\pm$ 11	376 $\pm$ 30	0.77 $\pm$ 0.04
UKU99	3.3 $\pm$ 1.6	13.6 $\pm$ 1.8	< 1	148 $\pm$ 25 ^c	408 $\pm$ 40	0.36 $\pm$ 0.05 ^c
UKU2	68.1 $\pm$ 22.7 ^{c,d}	5.2 $\pm$ 1.5 ^j	29 $\pm$ 12	209 $\pm$ 36	367 $\pm$ 35	0.58 $\pm$ 0.15
UKU2/UKU99	63.0 $\pm$ 14.6 ^{c,d}	10.0 $\pm$ 4.6	< 1	159 $\pm$ 21 ^c	399 $\pm$ 40	0.40 $\pm$ 0.10

Figure 3 Northern-blot analysis of brain RNA from transgenic (+tg) mice strains UKU99, UKU101, UKU103, UKU104 and their non-transgenic (–tg) littermates

Samples of total RNA (15 mg) isolated from mouse brains were used for RNA blot hybridization. The blots were hybridized sequentially with probes for AdoMetDC (A) or with a probe for 28 S ribosomal RNA as a control for the relative amounts of RNA in the different lanes (B).

founders (results not shown), which also may explain the relatively low increases in the expression of the transgene.

ODC and AdoMetDC activities and polyamine concentrations in four tissues of AdoMetDC-overexpressing transgenic mice (UKU99) and their non-transgenic littermates are shown in Table 2. In all tissues tested, the AdoMetDC activities are higher in the transgenic animals than in their non-transgenic littermates but the relative increases vary between different tissues, brain displaying the largest increase (3.3-fold) and heart the smallest (1.5-fold). However, the results in Figure 2 show that there is a good correlation between the expression of the mouse AdoMetDC gene and the rat transgene in brain, heart, liver and testis ($r = 0.960$). These data further suggest that the construct used to produce transgenic mice contains *cis*-elements necessary to determine tissue specificity. In contrast, previous works on transgenic mice harbouring several copies of the human ODC gene in their genome revealed 10–100-fold overexpression of ODC activity in mouse tissues [12]. This expression, however, did not conform to the normal tissue distribution of the ODC activity, an aberrant expression being most strikingly manifested in the testis and brain. The transgene construct used to generate

ODC-overexpressing mice contained only an 800 bp 5'-flanking region, and therefore it is possible that tissue-specific silencer elements were missing in the transgene construct [12].

In the tissues of AdoMetDC-over-producing mice, no statistically significant changes were found in ODC activity relative to the activities in those of the non-transgenic littermates (Table 2). This could be anticipated, because tissue spermine levels were found to be practically unaltered and there were no marked changes in the tissue spermidine pools. However, the molar ratio of spermidine to spermine was found to be lower in all the tissues of transgenic mice relative to those of the control mice, resulting apparently from the rapid conversion of putrescine and spermidine into spermine in the presence of excess dcAdoMet. On the other hand, in AdoMetDC overexpressing cell lines, Chinese-hamster ovary/644 cells [31] or mouse FM3A cells [32], ODC activity is unchanged or even slightly increased, which is unexpected because increases in spermine pools observed in these cell lines typically results in down-regulation of the enzyme [33–35]. In these cell lines, increases in spermine levels, which are significant in contrast with the transgenic mice, occur at the cost of spermidine.

Previous studies have shown that rat and mouse AdoMetDC mRNAs have identical sizes (i.e. approx. 3.1 kb and 2.0 kb) [30,36]. Northern-blot analysis of AdoMetDC mRNA from the tissues of transgenic mice and their non-transgenic littermates showed that the mRNA of approx. 3.1 kb is dominant. The results on brain tissue are shown in Figure 3. Densitometric scanning of the AdoMetDC mRNA bands revealed that the increases in the brain enzyme activity paralleled the increases in AdoMetDC mRNA. This was also apparent in testis, spleen and heart (results not shown). It has been shown that the translation of AdoMetDC mRNA is negatively regulated by spermidine and spermine, possibly through a 21-nucleotide open reading frame in the 5'-untranslated region [34,37]. However, because tissue spermidine levels tend to decrease and spermine levels are practically unaltered (Table 2), translational suppression by polyamines is not expected to depress AdoMetDC expression in the over-producing mice.

Putrescine is depleted in AdoMetDC-over-producing cells [31,32,35], and this tendency is also obvious in the tissues of AdoMetDC transgenic mice (Table 2). To study whether the decreased availability of putrescine is the reason that spermidine

Table 3 Polyamine concentrations in transgenic and non-transgenic primary fibroblast cultures

Cells were grown to 60–80% confluence, fresh medium was added and cultures were harvested after 4 or 24 h. Results are means $\pm$ S.D. for at least four independent measurements. Significance of the differences between transgenic and non-transgenic: ^a $P < 0.02$, ^b $P < 0.05$.

	Polyamine concentration (pmol/ 10^6 cells)					
	Putrescine	Spermidine	Spermine	N ⁶ -Acetylspermidine	N ¹ -Acetylspermidine	N ¹ -Acetylspermine
Control 4 h						
Cells	770 $\pm$ 263	4272 $\pm$ 1222	3538 $\pm$ 255	< 1	31.1 $\pm$ 12.7	82.4 $\pm$ 55.1
Medium	447 $\pm$ 167	94.1 $\pm$ 36.4	11.5 $\pm$ 4.9	< 1	67.7 $\pm$ 25.4	6.8 $\pm$ 2.4
Hybrid 4 h						
Cells	1305 $\pm$ 193 ^a	6338 $\pm$ 2021	3210 $\pm$ 846	< 1	35.5 $\pm$ 18.1	83.1 $\pm$ 54.1
Medium	385 $\pm$ 132	49.4 $\pm$ 16.8	3.9 $\pm$ 1.0	< 1	61.6 $\pm$ 16.5	4.8 $\pm$ 2.6
Control 24 h						
Cells	2099 $\pm$ 558	7841 $\pm$ 2149	2420 $\pm$ 726	< 1	16.3 $\pm$ 6.8	99.8 $\pm$ 49.6
Medium	721 $\pm$ 199	58.0 $\pm$ 22.3	< 1	< 1	123.2 $\pm$ 89.3	< 1
Hybrid 24 h						
Cells	2309 $\pm$ 1163	5445 $\pm$ 1942	2824 $\pm$ 294	< 1	37.7 $\pm$ 15.7	61.7 $\pm$ 16.1
Medium	1710 $\pm$ 529 ^b	33.4 $\pm$ 29.9	41.2 $\pm$ 61.7	< 1	525.8 $\pm$ 175.2 ^b	8.7 $\pm$ 5.6

Table 4 Pulse labelling of polyamine flux in transgenic primary fibroblasts and non-transgenic fibroblasts

Cells were grown in Dulbecco's modified Eagle's medium/20% foetal-bovine serum, 37 °C. After 16 h (60–80% confluence) the medium was replaced with fresh medium containing 2 μ Ci of L-[U-¹⁴C]ornithine. After 2 h of labelling, the medium was removed, the cells were washed three times with PBS and fresh medium was added. Cells were grown for 4 or 24 h and the radioactivity in the polyamines and their acetyl derivatives was determined. Radioactivity is expressed as a percentage of the total radioactivity in the polyamine pool. Results are means $\pm$ S.D. for five independent measurements. Significance between transgenic and non-transgenic: ^a P < 0.05, ^b P < 0.01, ^c P < 0.001.

	Distribution of radioactivity (%)				
	Putrescine	Spermidine	Spermine	N ¹ -Acetylspermidine	N ¹ -Acetylspermine
Control 4 h					
Cells	86.2 $\pm$ 18.6	3.3 $\pm$ 2.4	4.8 $\pm$ 2.3	0	5.7 $\pm$ 2.0
Medium	84.9 $\pm$ 19.6	4.6 $\pm$ 2.8	3.1 $\pm$ 2.3	5.3 $\pm$ 2.7	2.1 $\pm$ 1.0
Hybrid 4 h					
Cells	14.7 $\pm$ 8.4 ^c	38.0 $\pm$ 8.4 ^c	8.8 $\pm$ 3.7	16.0 $\pm$ 9.6	22.5 $\pm$ 11.7 ^a
Medium	58.7 $\pm$ 22.8	6.9 $\pm$ 6.1	17.4 $\pm$ 13.7	13.0 $\pm$ 7.1	4.0 $\pm$ 2.5
Control 24 h					
Cells	67.2 $\pm$ 43.1	6.2 $\pm$ 2.7	11.1 $\pm$ 10.3	4.7 $\pm$ 2.4	10.8 $\pm$ 6.6
Medium	50.1 $\pm$ 17.2	4.8 $\pm$ 2.0	10.8 $\pm$ 9.2	27.6 $\pm$ 12.8	6.7 $\pm$ 4.5
Hybrid 24 h					
Cells	23.5 $\pm$ 10.9	19.6 $\pm$ 6.1 ^b	13.2 $\pm$ 8.8	18.2 $\pm$ 9.9 ^a	25.5 $\pm$ 9.4
Medium	28.4 $\pm$ 10.8	3.3 $\pm$ 3.3	13.0 $\pm$ 4.1	51.3 $\pm$ 13.8	4.0 $\pm$ 2.5

and spermine do not accumulate in the tissues of transgenic mice, we cross-bred transgenic mice that overexpress the ODC gene (UKU2) [12–14] and AdoMetDC transgenic mice (UKU99). Polyamine concentrations and AdoMetDC and ODC activities in brain, spleen and heart are shown in Table 2. In the hybrid transgenic mice, putrescine concentrations in the selected tissues tended to normalize relative to those in transgenic mice overexpressing ODC alone (UKU2), yet without any marked changes in the tissue spermidine or spermine pool.

The ability of the transgenic mice to maintain polyamine homeostasis in their tissues is not unexpected, because an excessive polyamine accumulation has been shown to be toxic [38] and to induce apoptosis [39]. To study the mechanisms by which polyamine homeostasis is maintained in hybrid transgenic mice, we performed pulse-labelling of polyamine flux for transgenic and non-transgenic primary fibroblasts with the ¹⁴C-labelled polyamine precursor, L-ornithine. The synthesis and secretion of higher polyamines were monitored by measuring the intracellular and extracellular polyamine concentrations and the incorporation of radioactivity in the polyamines and their acetyl derivatives. In agreement with the data of tissue polyamine concentrations, polyamine levels did not differ markedly between these two cell lines (Table 3). Intracellular putrescine concentration was found to be slightly higher in transgenic fibroblasts 4 h after addition of fresh medium than in control cells, and after 24 h more putrescine was excreted into the medium compared with the control. After 4 h there were no differences in the concentrations of acetylated polyamines between the two cell lines, but after 24 h the N¹-acetylspermidine concentration was higher in the medium of transgenic fibroblasts. However, pulse-labelling of polyamine flux (Table 4) shows that polyamine synthesis is faster in transgenic fibroblasts than in non-transgenic cells. Although we were not able to demonstrate any marked increase in the activity of spermidine/spermine N¹-acetyltransferase in the tissues of hybrid mice (results not shown), pulse labelling revealed increased acetylation of intracellular spermidine and spermine in transgenic fibroblasts. This suggests, together with the finding of higher concentrations of N¹-acetylspermidine in the medium, that the compensation mechanism that prevents accumulation of higher polyamines involves, at

least partly, acetylation and increased export out of the cell. Whether this adaptation also involves sequestration of the polyamines [5] so that they are not available for synthesis remains to be established.

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