Activation of Polyamine Catabolism Profoundly Alters Tissue Polyamine Pools and Affects Hair Growth and Female Fertility in Transgenic Mice Overexpressing Spermidine/Spermine N\(^1\)-Acetyltransferase*

(Received for publication, February 3, 1997, and in revised form, April 21, 1997)

Marko Pietilä‡, Leena Alhonen§, Maria Halmekytö‡, Peter Kanter§, Juhani Jänne§, and Carl W. Porter§

From the ‡A.I. Virtanen Institute, University of Kuopio, P.O. Box 1627 FIN-70211, Kuopio, Finland and the §Grace Cancer Drug Center, Roswell Park Cancer Institute, Buffalo, New York 14263

We have generated a transgenic mouse line that overexpresses the rate-controlling enzyme of polyamine catabolism, spermidine/spermine N\(^1\)-acetyltransferase. Tissues of these mice showed markedly distorted polyamine pools, which in most cases were characterized by the appearance of N\(^1\)-acetylsperrmidine, not normally found in mouse tissues, a massive accumulation of putrescine, and decreases in spermidine and/or spermine pools. The most striking phenotypic change was permanent hair loss at the age of 3 to 4 weeks which was typified histologically by the appearance of extensive follicular cysts in the dermis. The effect seemed attributable to putrescine interference with hair development, possibly with differentiation/proliferation of epidermal cells located in hair follicles. Female members of the transgenic line were found to be infertile apparently due to ovarian hypofunction and hypoplastic uteri. The findings demonstrate the utility of spermidine/spermine N\(^1\)-acetyltransferase overexpression as an effective means for genetically modulating total tissue polyamine pools in transgenic animals and examining the developmental and oncogenic consequences.

The well recognized association of polyamines with cell growth (1–3) is best illustrated by findings related to the key polyamine biosynthetic enzyme, ornithine decarboxylase (ODC).\(^1\) Although ODC is sharply but transiently increased by growth stimuli, it is constitutively activated during cell transformation induced by carcinogens, viruses, or oncogenes. Overexpression of ODC has been correlated with increased proliferative potential (4), tissue invasiveness (5), and in certain cell types, with oncogene-like transforming capabilities (6–8). Thus far, ODC appears to be the only growth-related gene activated by the transcription factors c-myc (9–11) and n-myc (12), suggesting a critical role for the enzyme in growth control. However, as indicated below, findings obtained in cell culture may not be directly applicable to conditions prevailing in vivo.

To define the role of polyamines in proliferative processes associated with the whole animal, we have generated a number of transgenic mouse and rat lines that overexpress ODC and/or other polyamine biosynthetic enzymes. Given the importance of polyamine biosynthetic activity to cell growth, the phenotypic changes were unexpectedly mild. In transgenic mice overexpressing ODC, the most marked effect was inhibition of meiotic DNA synthesis during spermatogenesis (13) ultimately leading to male infertility (14). It is also noteworthy that lifelong overexpression of ODC in mouse tissues did not seem to increase the incidence of spontaneous tumors (15). The absence of more profound phenotypic changes in these mice may be attributable to the relatively minor changes observed in higher polyamine pools. Despite severalfold increases in ODC activity, polyamine pool disturbances were mainly confined to putrescine accumulation, and the pools of those polyamines considered to be more significantly involved in cell growth, spermidine and spermine, were only minimally affected. We presume this is due to compensatory responses since in vitro systems indicate that polyamine pools are sensitively maintained by complex regulatory responses involving polyamine biosynthesis, catabolism, and transport (1). The existence and effectiveness of these responses in minimizing polyamine pool effects in the ODC transgenics further indicate the cellular importance of polyamines.

In this present study, we have undertaken an alternative approach to studying the whole animal consequences of polyamine pool perturbations, that of polyamine pool depletion. Because genetic inactivation of ODC is known from in vitro systems to be incompatible with cell growth (16), gene knock-out was considered untenable. Instead, we attempted lowering intracellular polyamine pools by overexpressing an enzyme regarded as rate-limiting in polyamine catabolism, spermidine/spermine N\(^1\)-acetyltransferase (SSAT). By acetylating these higher polyamines, the enzyme facilitates their back-conversion to putrescine and promotes their excretion out of cells (17). The net effect of these two processes is a reduction in the higher polyamine pools, an event presumably intended to prevent cellular toxicity due to polyamine excess (1).

Very little is known about the metabolic consequences resulting from constitutive overexpression of SSAT. Recombinant human SSAT has been efficiently expressed in Escherichia coli using an inducible expression vector (18). In induced cells, all of the cellular spermidine was rapidly converted to the SSAT product, N\(^1\)-acetylspermidine, resulting in a reduction in cellular growth rate (18). Attempts to constitutively overexpress SSAT in mammalian cells have been unsuccessful due to poor

---

\(^1\) The abbreviations used are: ODC, ornithine decarboxylase; AdoMet, S-adenosylmethionine; SSAT, spermidine/spermine N\(^1\)-acyetyltransferase; kbp, kilobase pair(s); PCR, polymerase chain reaction.
Activated Polyamine Catabolism in Transgenic Mice

EXPERIMENTAL PROCEDURES

Generation of Transgenic Mice—The transgenic mice were generated using the standard pronuclear microinjection technique (23). Fertilized oocytes were obtained from superovulated BALBc × DBA/2 (CD2F1) females mated with CD2F1 males.

Gene Construct Used—The gene construct used was a 18-kbp genomic sequence isolated from 129 SVJ mouse genomic library in the lambda Fix® vector (Stratagene, La Jolla, CA). The library was screened with a polymerase chain reaction (PCR) probe amplified from reverse-transcribed mouse brain RNA. The probe covered nucleotides 137–744 of mouse (Mus domesticus) SSAT cDNA (24). The transgene construct contained all the exons and introns of the SSAT gene together with 3 kbp of the 5′-flanking region and 11.5 kbp of the 3′-flanking region.

Detection of the Transgene—As the transgene construct was an endogenous mouse gene, the method for its detection was based on the integration of multiple transgene copies in the form of concatamers (head-to-tail) so that a minimum of two integrated transgene copies were required for the detection. The design of the oligonucleotides was based on the head-to-tail recombination of the transgenes so that one primer recognized the tail region and one the head region of the SSAT gene. The following oligonucleotide primers were used in the PCR: 5′-GGTCCTTCTTGGCAGCAATAGGGC (from 3′ tail) and 5′-CAGGAAATTAC-AG-GCAAACCAAC-3′ (from 5′ head).

Analytical Methods—DNA was isolated by the method of Blin and Stafford (25). Total RNA was extracted with guanidine isothiocyanate (26) and purified by CsCl gradient centrifugation (27). In Northern and Southern blot analyses, human SSAT cDNA probe (28) (GenBank accession number L10244) or that generated for the library screening (see above) were used. Dot blot analysis with serially diluted kidney DNA samples was used to determine the gene copy number. To study the expression of mouse hairless gene (29), a 596-nucleotide PCR product covering nucleotides 1142–1740 in the third exon of the gene (29) was amplified and used as a probe in Northern blot analyses. SSAT, ODC, and AdoMet decarboxylase activities were assayed as described earlier (30). In initial studies (Table 1), SSAT activity was expressed as pmol of N\(^1\)-acetyl spermidine generated per min/mg of tissue and in later studies (Table II) as pmol of N\(^1\)-acetyl spermidine/min/mg protein. Polyamines and the acetylated derivative of spermine were measured by high pressure liquid chromatography as described by Kramer et al. (31). For statistical analyses, two-tailed t test was used. The tissue specimens for histology were fixed in 10% formalin in phosphate buffer, embedded in paraffin, cut into 5-μm sections, and stained with hematoxylin/eosin. Tissues of the mice were carefully examined grossly and histologically for changes by veterinary pathologists.

RESULTS

Gene Copy Numbers in the Transgenic Animals—We generated a female founder animal (169F\(_0\)) harboring a large number of copies of the SSAT gene. Phenotypically this animal was hairless from early age, extremely small, and infertile. The second founder animal (165F\(_0\)) was a male that gives rise to two different kinds of offspring, animals with normal hair (line 165a) and animals that permanently lost their hair at the age of 3 to 4 weeks (line 165b). Southern blot analysis (HindIII digestion) of DNA extracted from kidneys of the transgenic animals and their syngenic littermates is depicted in Fig. 1. The endogenous mouse SSAT gene gave rise to at least three signals of different sizes. The main fragments common to all strains were about 7 and 4 kbp in size. The third HindIII fragment was larger and displayed a strain difference between BALB/c and DBA/2 (Fig. 1). The transgene-derived fragment was about 9 kbp in size (the size difference in comparison with the endogenous gene is based on the head-to-tail recombination of the transgene copies) and gave a signal in tissues of line 165a equivalent to a copy number of 2 to 5. By contrast, the members of line 165b harbored at least 20 copies of the transgene and the founder animal 169F\(_0\) even more (Fig. 1). A comparison of the digestion pattern of lines 165a and 165b indicated that the founder animal 165F\(_0\) apparently had two different sites of transgene integration as follows: one locus with only a few copies (inherited by the offspring of the line 165a), and the other with a large number of gene copies (inherited by the offspring of line 165b).

Expression of the Transgene—The mRNA levels in the liver and brain of the above transgenic animals is shown in Fig. 2. As indicated, the expression of SSAT in line 165a was only modestly increased and relative to syngenic animals, whereas expression in line 165b and especially the founder animal 169F\(_0\) showed strikingly elevated SSAT mRNA levels. Brain and liver of the founder animal 165F\(_0\) and two members of the line 165a and of 165b were also analyzed for SSAT activity and polyamine pools. As shown in Table I, the SSAT activity was enhanced 8- and 30-fold in liver and brain, respectively, of the founder animal 169F\(_0\), whereas no changes were seen in the members of line 165a, the offspring that carried the fewest transgenes. In the liver, but not in brain, of line 165b SSAT activity was distinctly enhanced. Consistent with these SSAT
activities, the polyamine pools in members of line 165a were practically unchanged, and those of the founder animal 169F₀ were drastically altered. Interestingly, the latter changes were clearly tissue-specific. The brain of the 169F₀ animal displayed a moderate accumulation of putrescine, a massive increase in acetyl spermidine, and decrease in spermine but not spermidine. By contrast, the liver showed a massive accumulation of putrescine, a slight increase in spermidine, and a large decrease in spermine (Table I). The changes in the liver of the line 165b resembled those in the founder animal. We next undertook a detailed analysis of the members of the line 165b, the offspring that carried >20 copies of the transgene.

Characterization of the 165b Line—Fig. 3 shows the SSAT mRNA levels in selected tissues of the transgenic line 165b and their nontransgenic littersmates. In each of the tissues of transgenic animals, the mRNA levels of SSAT were distinctly elevated. It is obvious that the SSAT gene gives rise to two different messages. On the basis of findings in human cell lines (32, 33), the larger one probably represents preprocessed primary (heteronuclear) transcript. Table II shows the activities of SSAT, ODC, and AdoMet decarboxylase as well as the intracellular polyamine pools. Even though the SSAT activity was elevated in all tissues studied, the enhancement of the enzyme activity over that of the nontransgenic tissues was less than expected on the basis of mRNA levels (Fig. 3). This may be attributable to feedback control of SSAT activity by the polyamine pools via posttranscriptional mechanisms (28, 33). This view is also supported by the profound distortion of the polyamine pools indicative of a marked activation of the backconversion pathway in the transgenic animals.

In all of the tissues studied, there was a significant putrescine accumulation that varied according to tissue, from ~2-fold in the testes to ~25-fold in the spleen. The metabolic intermediate of the polyamine back-conversion pathway, N⁰-acetyl spermidine, was not found in the tissues of syngenic littersmates but accumulated to significant levels in all transgenic tissues (Table II). Neither N⁰-acetyl spermine nor N⁰-acetyl spermine was observed. With the exception of uterus, ovary, and testes, most of the transgenic tissues showed reduced spermidine and/or spermine pools. Markedly elevated ODC and AdoMet decarboxylase activities in most of the transgenic tissues probably reflect enzyme up-regulation by lowered spermidine and/or spermine pools. This interpretation was complicated, however, by the fact that the enzymes were also increased in ovary, uterus, and testes where polyamine pools were not decreased. Although the activity of ODC was substantially elevated in many tissues of the transgenic animals, it is unlikely that this rise contributed to the putrescine overaccumulation to any appreciable degree, a view that is supported by a close and highly significant correlation between putrescine accumulation and SSAT activity but not between putrescine and ODC activity (analysis not shown).

Phenotypic Changes—Of the 18 tissues subjected to a macroscopic and microscopic pathology evaluation, only the skin and organs of the female reproductive tract were found to be affected in the transgenic animals. As mentioned earlier, the founder animal 169F₀ was hairless from an early age, whereas members of the 165b line (having the larger number of SSAT gene copies of the two offspring lines) acquired normal first hair but lost it at the age of 3 to 4 weeks and remained hairless thereafter. Upon aging, the hairless skin of the transgenic animals appeared excessive and became extremely wrinkled as shown in Fig. 4. As indicated in Fig. 5, normal hair follicles (Fig. 5A) were replaced in the transgenic animals (Fig. 5B) by large cysts filled with keratin-like material. No hair shafts were visible. An occasional thickening of the epidermis was also seen in the skin of the transgenic animals, and subcutaneous fat cells were consistently much less apparent (Fig. 5A, 5B).

![Figure 2. Northern blot analysis of liver and brain SSAT mRNA (10 µg) obtained from nontransgenic (Co) and transgenic (165aF₁, 165bF₁, and 169F₀) mice. The migration of 18S rRNA is shown on the right.](image)

TABLE I

Comparison of polyamine metabolism in selected tissues from SSAT founder animal and related transgenic (tg) mouse lines

| Mouse   | Tissue | SSAT Activity<sup>a</sup> | Polyamine pools | Putrescine | Ac-spermidine<sup>b</sup> | Spermidine | Spermine |
|---------|--------|--------------------------|----------------|------------|--------------------------|------------|----------|
|         |        | fold-change | pmol/mg protein |            |             |            |          |
| 169F₀   | Brain  | <5          | 275           | 255        |
|         |        | >50         | 1400          | 750        |
|         | Liver  | >50         | 1010          | 770        |
|         |        | 5           | 70            | 1290       |
|         |        | 25          | 75            |
| 165aF₁  | Brain  | 2–5         | 285           | 235        |
|         |        | 0.84        | 251           | 220        |
|         | Liver  | 2–5         | 730           | 565        |
|         |        | 0.91        | 820           | 630        |
|         |        | 125         | 565           |
| 165bF₁  | Brain  | >20         | 221           | 292        |
|         |        | 0.97        | 221           | 292        |
|         | Liver  | >20         | 801           | 752        |
|         |        | 10.6        | 872           |

<sup>a</sup> Activity was initially measured as pmol/mg tissue and then converted to fold-change. The latter may underestimate increases in enzyme activity since in addition to SSAT activity, the assay measures other acetyltransferases in addition to SSAT.

<sup>b</sup> Ac-spermidine, N⁰-acetyl spermidine.
bility of genetically modulating tissue polyamine pools by over-
expression of a polyamine catabolic enzyme. Our previous exper-
iments with transgenic animals overexpressing genes of the
biosynthetic enzymes of the polyamines (9, 10) revealed that
spuridine and spermine pools are remarkably resistant to
depletion. Despite the availability of large quantities of the pre-
cursor putrescine in the ODC transgenic mice, the diamine
was not converted to these higher polyamines (34). As shown here,
expression of SSAT produces much more profound changes
in the polyamine pools. Although massive accumulation of pu-
rescine was also a very prominent feature, significant lowering
of spermidine and spermine pools also took place. While in
ODC transgenics, putrescine accumulation was obviously due
to increased decarboxylation of ornithine, the accumulation
of putrescine in the SSAT transgenics derives largely from a
metabolic disassembly of the higher polyamines. The unusual
appearance in transgenic tissues of significant quantities of N1-
acetyl spermidine, a metabolic intermediate in the back-conver-
sion pathway, is entirely consistent with this interpretation.
The present findings illustrate for the first time the meta-
bolic consequences of sustained overexpression of SSAT
activity in a mammalian system. In the context of the intact mouse,
the present results indicate that the effects of SSAT expression
were clearly tissue-dependent. Despite identical gene copy
numbers and similar transcript levels (Figs. 1 and 3), the levels
of expressed SSAT activity frequently differed among tissues and
tended to follow those of the basal levels for a tissue. This is
consistent with our previous findings indicating that a large
proportion of SSAT expression is regulated via polyamine-me-
diated mechanisms affecting translation of the enzyme protein
(33). Thus, the SSAT enzyme activity profiles observed in the
transgenic animals represent an established equilibrium of life-long homeostatic adjustments between that enzyme, poly-
amine pools, biosynthetic enzymes, and polyamine uptake.
Despite compensatory responses, tissue levels of polyamine
pools were significantly but differentially affected by SSAT

**DISCUSSION**

One goal of the present study was to investigate the feasi-
bility of genetically modulating tissue polyamine pools by over-
expressing a polyamine catabolic enzyme. Our previous exper-
iments with transgenic animals overexpressing genes of the

![Table II: Tissue polyamine metabolism in SSAT transgenic (tg) mouse line 165b](image)

| Tissue       | Enzyme activity | Polyamine pools | pmol/mg tissue |
|--------------|-----------------|-----------------|----------------|
|              | SSAT            | ODC             | AdoMetDC       | Putrescine | Ac-Spermidine | Spermidine | Spermine |
| Liver tg −   | 7 ± 6           | 12 ± 2          | 447 ± 392      | 15 ± 7     | <5           | 1098 ± 228 | 794 ± 36  |
| Liver tg +   | 29 ± 6**        | 256 ± 34**      | 632 ± 298      | 317 ± 94** | 67 ± 13***   | 1112 ± 133 | 330 ± 8***|
| Kidney tg −  | 9 ± 6           | 69 ± 39         | 57 ± 17        | 20 ± 9     | <5           | 302 ± 31   | 645 ± 57  |
| Kidney tg +  | 24 ± 13         | 275 ± 96**      | 107 ± 38       | 154 ± 14***| 20 ± 3***    | 262 ± 14   | 510 ± 29* |
| Lung tg −    | 4 ± 2           | 5 ± 4           | 14 ± 3         | 18 ± 6     | <5           | 410 ± 122  | 306 ± 106 |
| Lung tg +    | 17 ± 5*         | 11 ± 9          | 89 ± 17**      | 814 ± 280**| 53 ± 34      | 240 ± 67   | 223 ± 68  |
| Spleen tg −  | 57 ± 21         | 8 ± 4           | 152 ± 64       | 79 ± 27    | <5           | 1108 ± 226 | 842 ± 69  |
| Spleen tg +  | 89 ± 28         | 239 ± 93*       | 342 ± 49*      | 2087 ± 428*| 261 ± 140*** | 785 ± 225  | 675 ± 224 |
| Thymus tg −  | 10 ± 6          | 26 ± 6          | 65             | 114        | <5           | 930 ± 478  | 487       |
| Thymus tg +  | 23 ± 16         | 275 ± 155       | 106 ± 55       | 1227       | 40           | 475 ± 333  | 333       |
| Small intestine tg − | 37 ± 25       | 173 ± 141       | 79 ± 33        | 34 ± 44    | <5           | 1249 ± 25  | 689 ± 67  |
| Small intestine tg + | 77 ± 32       | 1850 ± 1597     | 434 ± 85**     | 732 ± 186**| 50 ± 18**    | 522 ± 141***| 448 ± 88* |
| Skin tg −    | 6 ± 4           | 26 ± 22         | 15 ± 7         | 51 ± 9     | <5           | 286 ± 64   | 166 ± 53  |
| Skin tg +    | 14 ± 5          | 6 ± 2           | 739 ± 75***    | 281 ± 37** | 26 ± 2***    | 272 ± 24   | 113 ± 14  |
| Heart tg −   | 0.5 ± 0.4       | 25 ± 3          | 25 ± 10        | 9 ± 2      | <5           | 143 ± 14   | 272 ± 16  |
| Heart tg +   | 1.5 ± 0.9       | 81 ± 46         | 29 ± 8         | 74 ± 4***  | 6 ± 1***     | 55 ± 8***  | 201 ± 14***|
| Brain tg −   | 8 ± 4           | 2 ± 2           | 117 ± 67       | 7 ± 0.3    | <5           | 281 ± 38   | 242 ± 37  |
| Brain tg +   | 13 ± 3          | 20 ± 11         | 164 ± 40       | 161 ± 23*  | 13 ± 2***    | 205 ± 68   | 241 ± 58  |
| Testes tg −  | <5             | 153 ± 103       | 30             | <5         | 272 ± 428    | 64 ± 39    | 450       |
| Testes tg +  | 6              | 198 ± 205       | 83             | 17         | 340 ± 170    | 134 ± 92   | 75        |
| Ovary tg −   | 11             | 26 ± 51         | 10             | <5         | 134 ± 92    | 128 ± 75   | 75        |
| Ovary tg +   | 28             | 335 ± 151       | 98             | 5          | 340 ± 170    | 134 ± 92   | 75        |
| Uterus tg −  | 6              | 28 ± 57         | 33             | <5         | 220 ± 100    | 134 ± 92   | 75        |
| Uterus tg +  | 55             | 259 ± 256       | 510           | 24         | 286 ± 146    | 134 ± 92   | 75        |

* Data are mean value ± S.D. where n = 3. Figures without deviations indicate that a pooled sample from three animals has been analyzed.

** Enzyme activities expressed as: SSAT, pmol/min/mg protein; ODC and AdoMetDC, pmol/hr/mg protein.

*** Ac-Spermidine, N1-acetyl spermidine.
overexpression. While all tissues showed increased levels of putrescine, not all displayed decreased levels of spermidine and spermine. Interestingly, those tissues that most effectively resisted changes in these higher polyamines tended to be those associated with reproduction (i.e. testes, ovary, and uterus). The biological significance of these interesting trends is not immediately apparent. The possibility exists that at an earlier time in development the polyamine pools were much more affected. The brain emerged as the only tissue in which spermidine was lowered but spermine was not, whereas liver was the only tissue in which spermine was lowered but spermidine was not, perhaps because this organ is especially active in AdoMet metabolism. In almost all tissues ODC and/or AdoMet decarboxylase were up-regulated to a level which, in most cases, again seemed to be determined by the basal enzyme activity of a particular tissue. It is presumed that this increase in enzyme is a compensatory response to SSAT lowering of spermidine and spermine pools (1). In tissues where polyamine pools are not lowered but enzymes were up-regulated, such as in the ovary and uterus, it is difficult to assign such cause and effect relationships since pools may have been low at an earlier time but now appear normal due to the regulatory increases in the biosynthetic enzymes. Obviously, the metabolic basis for the observed tissue polyamine profiles is too complex to allow a full interpretation based on the current data. The various regulatory responses to SSAT overexpression could probably be better understood following short term induced expression of the enzyme as opposed to examining the final results of long term homeostatic adjustments.

Regarding the phenotypic changes seen in the skin, the present animals resemble the hairless mutation of the mouse, an alteration caused by a proviral insertion (35) and disruption of the so-called hairless gene (29). The latter encodes a putative transcription factor expressed only in the skin and brain (29). This hairless mutation is recessive (35) and can be induced, in addition to proviral insertion, by an insertional inactivation by a transgene (36). We believe that that the hairless phenotype of our transgenic mice was not caused by insertional inactivation of the gene since the present phenotype is dominant instead of recessive and since we generated a founder animal and an independent transgenic line with exactly the same phenotype. In addition, we examined the expression of the hairless gene in our transgenic animals using a PCR-generated probe covering most of the third exon of the gene. By this analysis, hairless gene mRNA was present at a level only slightly below that of the nontransgenic mice (data not shown).

There is striking phenotypic similarity between our present transgenic mice and those overexpressing a stabilized ODC enzyme under skin-specific (keratin) promoter (37, 38). The timing of the hair loss, the wrinkling of the skin, and the histopathological changes were almost identical for the two transgenics. In this regard, it is somewhat surprising that overexpression of ODC, a gene expected to increase tissue polyamine levels, would give rise to a phenotype that is apparently identical to that seen with overexpression of SSAT, a gene expected to lower tissue polyamine pools. However, one prominent feature shared by both transgenics is the massive overaccumulation of putrescine in the skin. It seems likely, therefore, that this imbalance in skin putrescine pools may be responsible for shifting the differentiation process of the epidermal hair follicle cells to one of proliferation as has been proposed by O’Brien’s group (38). This is particularly interesting since putrescine is not typically implicated in proliferative processes in in vitro systems (1–3). The contribution of possible oxidative stress caused by polyamine catabolism should also be considered in the case of hair loss in the SSAT transgenics. In contrast to skin-specific ODC transgenics, there was no indication of skin papilloma formation (37) in the SSAT mice. Whether the absence of spontaneous tumor formation in the SSAT transgenic animals is related to the unaltered or slightly decreased skin pools of spermidine and spermine (Table II), in contrast to those elevated in ODC transgenic mice (37), is not known.

Even though the exact mechanism(s) leading to ovarian hypofunction and infertility of the female members of the present transgenic animals overexpressing SSAT is not known, it is of interest to note that a testicular overexpression of ODC in

**Fig. 4. Photograph of transgenic mouse of line 165b.** Note the absence of hair and the heavily wrinkled skin.

**Fig. 5. Histology of normal (A) and transgenic (B) skin (magnification 100 ×).** Note the replacement of hair follicles with large keratin containing cysts in the dermis of the transgenic mice (B). In addition, there is an absence of subcutaneous fat in the transgenic skin.
transgenic mice caused disturbances of spermatogenesis (13) ultimately leading to male infertility (14). Thus, as in the skin, increases in putrescine could play a role in mediating the effects on ovarian function.

In addition to revealing novel metabolic and phenotypic changes due to overexpression of SSAT, the present findings validate the approach for using the polyamine catabolic enzyme SSAT as a means to deplete intracellular spermidine and spermine pools in whole animals. This strategy can now be extended to conditional (39) and/or tissue-specific expression systems (37) to more specifically evaluate the physiological importance of these molecules in the biology of skin and other tissues.

Acknowledgments—We thank John Miller, Paula Diegelman, Gary Bullard, Tuula Reponen, and Jukka Korhonen for their excellent technical assistance and Barbara Holdridge for taking care of animal breeding.

REFERENCES
1. Porter, C. W., Regenass, U., and Bergeron R. J. (1992) in Falk Symposium on Polyamines in the Gastrointestinal Tract (Dowling, R. H., Folsch, U. R., and Loser, C., eds) pp. 301–322, Kluwer Academic Publishers Group, Drodrecht, Netherlands
2. Janne, J., Alhonen, L., and Leinonen, P. (1991) Ann. Med. 23, 241–259
3. Marton, L. J., and Pegg, A. E. (1995) Annu. Rev. Pharmacol. Toxicol. 35, 55–91
4. Sistonen, L., Keski-Oja, J., Ulmanen, I., Haltia, E., Wikgren, B.-J., and Alitalo, K. (1987) Exp. Cell Res. 168, 518–530
5. Polvinen, K., Sinervirta, R., Alhonen, L., and Janne, J. (1988) Biochem. Biophys. Res. Commun. 155, 373–378
6. Auvrin, M., Paasinen, A., Andersson, L. C., and Hölttä, E. (1992) Nature 360, 355–358
7. Moshier, J. A., Dosescu, J., Skunca, M., and Luk, G. D. (1993) Cancer Res. 53, 2618–2622
8. Hiibshoob, H., Johnson, M., and Weinstein, I. B. (1992) Oncogene 6, 739–743
9. Bello-Fernandez, C., Packham, G., and Cleveland, J. L. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 7804–7808
10. Wagner, A. J., Meyers, C., Laimins, L. A., and Hay, N. (1993) Cell Growth & Differ. 4, 879–883
11. Peña, A., Reddy, C. D., Wu, S., Hickok, N. J., Reddy, E. P., Yumet, G., Soprano, D. R., and Soprano, K. J. (1993) J. Biol. Chem. 268, 27277–27285
12. Lutz, W., Stühr, M., Schurmann, J., Wenzel, A., Lohr, A., and Schwab, M. (1996) Oncogene 13, 803–812
13. Hakovirta, H., Keiski, A., Toppari, J., Halmekyto, M., Alhonen, L., Janne, J., and Parvinen, M. (1993) Mol. Endocrinol. 7, 1430–1436
14. Halmekyto, M., Hyttinen, J.-M., Sinervirta, R., Utriainen, M., Myshahnen, S., Voipio, H.-M., Wahlfors, J., Syrjanen, S., Syrjanen, K., Alhonen, L., and Janne, J. (1991) J. Biol. Chem. 266, 19746–19751
15. Alhonen, L., Halmekyto, M., Kosma, V.-M., Wahlfors, J., Kauppinen, R., and Janne, J. (1995) Int. J. Cancer 63, 402–404
16. Pila, R. R., Steglich, C., and Scheffler, I. E. (1990) J. Biol. Chem. 265, 8880–8886
17. Casero, R. A., and Pegg, A. E. (1993) PASEB J. 7, 653–661
18. Parry, L., Lopez-Ballester, J., West, L., and Pegg, A. E. (1995) Biochemistry 34, 2701–2709
19. Parry, L., Balaña Fouce, R., and Pegg, A. E. (1995) Biochem. J. 305, 451–458
20. Vargiu, C., and Persson, L. (1994) FEBS Lett. 355, 163–165
21. Casero, R. A., Jr., Celano, P., Ervin, S. J., Porter, C. W., Bergeron, R. J., and Libby, P. R. (1989) Cancer Res. 49, 3829–3833
22. Shappell, N. W., Miller, J. T., Bergeron, R. J., and Porter, C. W. (1992) Anticancer Res. 12, 1083–1098
23. Hogan, B., Constantini, F., and Lacy, E. (1986) Manipulating the Mouse Embryo, pp. 1–322, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
24. Fogel-Petrovic, M., Kramer, D. L., Ganis, B., Casero, R. A., Jr., and Porter, C. W. (1993) Biochim. Biophys. Acta 1216, 255–264
25. Blin, N., and Stafford, D. W. (1976) Nucleic Acids Res. 3, 2303–2306
26. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
27. Ross, J. R. (1976) J. Mol. Biol. 106, 403–420
28. Fogel-Petrovic, M., Vujicic, S., Brown, P. J., Haddox, M. K., and Porter, C. W. (1996) Biochemistry 35, 14436–14444
29. Cachon-Gonzales, M. B., Fenner, S., Coffin, J. M., Moran, C., Best, S., and Stoye, J. P. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7717–7721
30. Bernacki, R. J., Oberman, E. J., Seweryniak, K. E., Atwood, A., Bergeron, R. J., and Porter, C. W. (1995) Clin. Cancer Res. 1, 847–857
31. Kramer, D., Mett, H., Evans, A., Regenass, U., Diegelman, P., and Porter, C. W. (1995) J. Biol. Chem. 270, 2124–2132
32. Fogel-Petrovic, M., Shappell, N. W., Bergeron, R. J., and Porter, C. W. (1993) J. Biol. Chem. 268, 19118–19125
33. Fogel-Petrovic, M., Vujicic, S., Miller, J., and Porter, C. W. (1996) FEBS Lett. 391, 89–94
34. Halmekyto, M., Alakuijala, L., Alhonen, L., and Janne, J. (1993) Biochem. Biophys. Acta 1216, 505–508
35. Stoye, J. P., Fenner, S., Greenoak, G. E., Moran, C., and Coffin, J. M. (1988) Cell 54, 383–391
36. Jones, J. M., Elder, J. T., Simin, K., Keller, S. A., and Meisler, M. H. (1993) Mamm. Genome 4, 639–643
37. Megosh, L., Gilmour, S. K., Rossen, D., Peralta Soler, A., Blessing, M., Sawicki, J. A., and O'Brien, T. G. (1995) J. Invest. Dermatol. 105, 1108–1113
38. Megosh, L., Gilmour, S. K., Rosson, D., Peralta Soler, A., Blessing, M., Sawicki, J. A., and O'Brien, T. G. (1995) J. Invest. Dermatol. 105, 1108–1113