Spermidine/spermine-\(N^1\)-acetyltransferase: a key metabolic regulator

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Submitted 6 February 2008; accepted in final form 5 March 2008

SSAT and its central role in polyamine metabolism and other cellular physiology. This review summarizes recent studies on alterations in SSAT have been linked to a variety of pathologi- cal conditions. This review summarizes recent studies on SSAT and its central role in polyamine metabolism and other cellular physiology.

SSAT is a key enzyme in these processes. SSAT is very highly regulated. Its content is adjusted in response to alterations in polyamine content to maintain polyamine homeostasis. Certain polyamine analogs can mimic the induction of SSAT and cause a loss of normal polyamines. This may have utility in cancer chemotherapy. SSAT activity is also induced via a variety of other stimuli, including toxins, hormones, cytokines, nonsteroidal anti-inflammatory agents, natural products, and stress pathways, and by ischemia-reperfusion injury. These increases are initiated by alterations in Sat1 gene transcription reinforced by alterations at the other regulatory steps, including protein turnover, mRNA processing, and translation. Transgenic manipulation of SSAT activity has revealed that SSAT activity links polyamine metabolism to lipid and carbohydrate metabolism by means of alterations in the content of acetyl-CoA and ATP. A high level of SSAT stimulates flux through the polyamine biosynthetic pathway, since biosynthetic enzymes are induced in response to the fall in polyamines. This sets up a futile cycle in which ATP is used to generate \(S\)-adenosylmethionine for polyamine biosynthesis and acetyl-CoA is consumed in the acetylation reaction. A variety of other effects of increased SSAT activity include death of ovarian cells, blockage of regenerative tissue growth, behavioral changes, keratosis follicularis spinulosa decalvans, and hair loss. These are very likely due to changes in polyamine and putrescine levels, although increased oxidative stress via the oxidation of acetylated polyamines may also contribute. Recently, it was found that the SSAT protein and/or a related protein, thialysine acetyltransferase, interacts with a number of other important proteins, including the hypoxia-inducible factor-1 \(\alpha\)-subunit, the p65 subunit of NF-\(B\), and \(\alpha\)9\(\beta\)1-integrin, altering the function of these proteins. It is not yet clear whether this functional alteration involves protein acetylation, local polyamine concentration changes, or other effects. It has been suggested that SSAT may also be a useful target in diseases other than cancer, but the wide-ranging physiological and pathophysiological effects of altered SSAT expression will require very careful limitation of such strategies to the relevant cell types.

polyamines; spermine; spermidine; acetyl-coenzyme A

SSAT is a key regulatory enzyme in polyamine metabolism. It catalyzes the acetylation of polyamines, which is a key step in regulating polyamine levels. SSAT activity is tightly regulated and can be induced by various stimuli, including toxins, hormones, cytokines, and stress pathways. This regulation is important for maintaining polyamine homeostasis and can have significant physiological and pathological consequences. SSAT activity is also induced via a variety of other stimuli, including toxins, hormones, cytokines, nonsteroidal anti-inflammatory agents, natural products, and stress pathways, and by ischemia-reperfusion injury. These increases are initiated by alterations in Sat1 gene transcription reinforced by alterations at the other regulatory steps, including protein turnover, mRNA processing, and translation. Transgenic manipulation of SSAT activity has revealed that SSAT activity links polyamine metabolism to lipid and carbohydrate metabolism by means of alterations in the content of acetyl-CoA and ATP. A high level of SSAT stimulates flux through the polyamine biosynthetic pathway, since biosynthetic enzymes are induced in response to the fall in polyamines. This sets up a futile cycle in which ATP is used to generate \(S\)-adenosylmethionine for polyamine biosynthesis and acetyl-CoA is consumed in the acetylation reaction. A variety of other effects of increased SSAT activity include death of ovarian cells, blockage of regenerative tissue growth, behavioral changes, keratosis follicularis spinulosa decalvans, and hair loss. These are very likely due to changes in polyamine and putrescine levels, although increased oxidative stress via the oxidation of acetylated polyamines may also contribute. Recently, it was found that the SSAT protein and/or a related protein, thialysine acetyltransferase, interacts with a number of other important proteins, including the hypoxia-inducible factor-1 \(\alpha\)-subunit, the p65 subunit of NF-\(B\), and \(\alpha\)9\(\beta\)1-integrin, altering the function of these proteins. It is not yet clear whether this functional alteration involves protein acetylation, local polyamine concentration changes, or other effects. It has been suggested that SSAT may also be a useful target in diseases other than cancer, but the wide-ranging physiological and pathophysiological effects of altered SSAT expression will require very careful limitation of such strategies to the relevant cell types.
increase in putrescine and a loss of spermidine and spermine in rodent liver. Studies with radioactive polyamines showed that these changes were due to an increased conversion of these higher polyamines into putrescine (63), but there was no increase in an oxidase that brought about this reaction. A cytosolic enzyme that acetylated polyamines was found to be highly inducible by carbon tetrachloride, and it was suggested that the acetylation products were the true oxidase substrate (93). Purification and characterization of this SSAT enzyme showed that it was quite distinct from other enzymes such as histone acetylases (which have some activity on polyamines) and that it acetylated only the N\textsuperscript{1} position of spermidine (the aminopropyl end; see Fig. 1B) (94). Spermine, which is symmetrical, could be acetylated at either end. More detailed examination of SSAT substrate specificity showed that a variety of substrates with the general structure \(H_2N(CH_2)_{n}NH\text{R}\), including \(\text{N}^1\)-acetylspermidine, \(\text{sym}\)-norspermine, and \(\text{sym}\)-norspermidine, are excellent substrates for SSAT, but putrescine, \(\text{N}^1\)-acetylspermine, and \(\text{sym}\)-homospermidine, which have terminal aminobutyl groups, are not substrates (41). These observations were recently confirmed with the additional information that diamines separated by two methylene groups such as diethylenetriamine can also be acetylated, although poorly (61).

SSAT is able to use \(\text{N}^1\)-acetylspermidine as a substrate forming \(\text{N}^1,\text{N}^{11}\)-diacetylspermine, and this product has been found in both cell extracts and culture medium from cells with high SSAT levels and in urine of mice overexpressing SSAT. This suggests that in vivo, \(\text{N}^1\)-acetylspermidine is more likely to be acetylated than spermine itself, but the \(K_m\) value for spermine is actually lower than that for \(\text{N}^1\)-acetylspermidine (41). A plausible explanation is that most of the cellular spermine is bound to membranes or nucleic acids and that a much higher percentage of the acetyl derivative is directly available to the soluble SSAT enzyme. It is also possible that the initial \(\text{N}^1\)-acetylspermidine product remains in the active site and is reoriented to allow a second acetyl transfer, as appears to be the case with the \emph{Bacillus subtilis} spermine acetyltransferase (141).

The presence of acetylated polyamines in urine or cell extracts is usually a reliable marker of increased SSAT activity, but their accumulation depends on their ability to escape degradation by APAO. APAO content can therefore also contribute to the content of acetylated polyamines. In breast tumors, it was found that APAO was decreased and SSAT increased, so the levels of both enzymes contributed to an increase in acetylated polyamines (131).

**Genetics.** Cloning of the SSAT cDNA (19, 145) and gene (144) were facilitated by the important discovery by Casero and colleagues that treatment of some human cancer cells such as NCI H157 human lung carcinoma cells with polyamine analogs \(\text{N}^1,\text{N}^{11}\)-bis(ethyl)spermine (BE-3-4-3) or \(\text{N}^1,\text{N}^{11}\)-bis(ethyl)norspermine (BE-3-3-3) leads to a very high induction of SSAT (20), providing a source for the isolation of the protein (21). Partial protein sequence analysis then allowed cloning of the gene. Human SSAT, which is a member of the GNAT (GCN5-related \(N\)-acyetyltransferase) family, is a 171-amino acid protein that is active as a homodimer. Very similar proteins occur in other vertebrates. Other enzymes acetylating polyamines have been described from \emph{B. subtilis} and \emph{Escherichia coli} (49, 141), yeast (85), and parasites (39, 146), but these differ from SSAT in structure and/or substrate specificity and are not considered in this review.

The Sat1 gene encoding SSAT is present on the X chromosome at location Xp22.1. It contains six exons; exon 1 contains the 5\textsuperscript{'}-untranslated retion (UTR) and the first 22 codons of the coding sequence; exon 6 contains the last 56 amino acid codons, the stop codon, and the 3\textsuperscript{'}-UTR (144) (Fig. 2A). These
exons encode mRNAs of about 1.3 and 1.5 kb [including poly(A)] (47). An alternative splice variant, which includes an additional 110-bp exon between exons 3 and 4, is also formed and provides an important regulatory mechanism, described below. The promoter region lacks a TATA box but has potential binding sites for multiple transcription factors, including Sp-1, AP-1, CCAT/enhancer binding protein-β (C/EBPβ), cAMP response element binding protein (CREB), NF-κB, and peroxisome proliferator-activated proteins (PPARs) (7, 31, 45, 144) (Fig. 2B).

DNA sequence analysis revealed that another GNAT family protein gene present in the human genome, termed Sat2, encodes a 170-amino-acid protein having 46% sequence identity and 64% sequence similarity with SSAT. The protein product of this gene was initially described as SSAT2 due to a very limited ability to acetylate polyamines in in vitro assays at high substrate concentrations (29), but more detailed characterization showed that the $k_{cat}/K_m$ specificity constant for spermidine and spermine was $<0.001\%$ that of genuine SSAT and that expression of SSAT2 in cells did not increase acetyl-polyamines or reduce free polyamine content (38). Human SSAT2 has a much greater activity toward the ε amino group of thialysine [S-(2-aminoethyl-l-cysteine)] (38) and is better described as thialysine-$N^\varepsilon$-acyltransferase (TLAT). TLAT is quite widely distributed; well-characterized examples are the TLATs from Caenorhabditis elegans (1), Schizosaccharomyces pombe (38), and Leishmania major (87). These enzymes efficiently acetylate thialysine, 5-hydroxy-L-lysine, S-(aminoethyl)-homocysteine, and O-(aminoethyl)-l-serine and have a modest activity on Lys itself. However, the physiological function of this acetylation is not understood.

Structure. The crystal structure of human SSAT was determined recently (15) (Fig. 3). A subsequent structure of SSAT bound to a bisubstrate analog (N1-spermine-acetyl-CoA) and accompanying kinetic observations have confirmed and advanced these observations (61). An important finding during the initial determination of the SSAT structure, which may have important implications for SSAT physiology and func-

Fig. 2. SSAT gene structure. A: SSAT gene transcript showing exons. Portions shown in blue correspond to the 5'- and 3'-untranslated region (UTR), respectively. Portions shown in red correspond to those including the amino acid codons. B: a 3.5-kb clone encompassing the SSAT gene region showing the position of proposed binding sites for transcription factors (7, 144). The transcription start site is marked. Transcription factor binding sites for AP-1, CCAT/enhancer binding protein-β (C/EBPβ), cAMP response element (CRE), NF-κB, peroxisome proliferator-activated protein repressor element (PPRE), and Sp-1 are shown, as is the polyamine responsive element (PRE).

Fig. 3. Structure of SSAT and thialysine-$N^\varepsilon$-acyltransferase (TLAT; or SSAT2). Top: the structures are shown as a ribbon diagram of SSAT (left) and TLAT (middle) and with both structures superimposed (right). Each structure is composed of a homodimer where each monomer is uniquely colored. Acetyl-CoA is colored in a stick representation. Only 1 acetyl-CoA is shown in the TLAT structure, but both structures have 2 active sites. Bottom: the molecular surface of SSAT (left) and TLAT (right) is shown, colored according to element (nitrogen, blue; oxygen, red; carbon, gray; sulfur, yellow), demonstrating the differences between these proteins in surface properties.
tion was the observation that SSAT can self-acetylate at Lys$^{26}$ (15). Analysis of the recombinant human protein produced at high concentrations in E. coli showed that half of the monomers had an N-acetyl group on this residue. Alteration of this Lys to Arg prevented this acetylation without affecting the ability to acetylate polyamine substrates.

SSAT is a homodimer, which in the symmetrical configuration described below has two active sites, each formed at the dimer interface from residues of both subunits. The structure determined by Bewley et al. (15) shows two conformations: a symmetric form with two open surface channels able to bind substrate or cofactor and an asymmetric form with only one such channel. These forms are probably in equilibrium in solution.

The residues interacting with acetyl-CoA and spermine (or BE-3-3-3) in the SSAT crystal structure are in good agreement with the results of site-directed mutagenesis studies. The sequence R$^{101}$FGIGS$^{107}$ and residues Arg$^{142}$ and Arg$^{143}$ were implicated in acetyl-CoA binding (34, 86). In the crystal structure, the pyrophosphate loop of acetyl-CoA interacts with the backbone amide nitrogens of these Gly residues, the side chain of Arg$^{101}$ binds to the pantetheine moiety, and the side chains of Arg$^{142}$ and Arg$^{143}$ interact with the 3′ phosphate. The polypeptide binding pocket contains the side chains of Met$^{39}$, Ile$^{85}$, Leu$^{128}$, Trp$^{84}$, and Trp$^{154}$, which form interactions with the polyamine alkane regions, and Glu$^{28}$, Glu$^{32}$, Asp$^{82}$, Glu$^{92}$, Asp$^{93}$, Glu$^{152}$, and Asp$^{160}$, which interact with the charged N positions of spermine. As expected from the structure, the mutations E152K and R101A inactivate SSAT (33). However, coexpression of these inactive SSAT mutants restores activity (34). This is consistent with the crystal structure, which shows that Arg$^{101}$ (part of the acetyl-CoA binding site) and Glu$^{152}$ (part of the polypeptide binding site) are contributed to the active sites by different monomers. As predicted from this, the double mutant R101A/E152K acts as a dominant negative (34), and this mutant protein has been used as a tool to investigate SSAT function.

The crystal structures revealed that Tyr$^{140}$, a totally conserved residue, is likely to serve as the general acid for protonation of the thiolate anion of acetyl-CoA. Alteration of this residue in the Y140F mutant greatly reduced activity (15). A general base that extracts the proton for the attacking acid$^{34}$, Ser$^{32}$, Thr$^{33}$, and Leu$^{130}$, which may be located in the active site and differ from the corresponding residues in SSAT (87). The $K_m$ for thialysine was modestly (3- to 5-fold) increased by mutants R31A and L130A, whereas mutants S82A and T83A led to a vast reduction in activity. Unfortunately, the published structures of TLAT contain only bound acetyl-CoA but no amine substrate and cannot be used to support the key role of these amino acids.

**Assay and inhibition of SSAT.** Under resting conditions, SSAT activity in the cell is very low. Attempts to measure this activity by using assays that measure acetylpolyamine production in the presence of acetyl-CoA invariably overestimate the activity due to nonenzymatic acetylation and the fact that proteins other than SSAT can carry out this reaction. Rigorous determination of SSAT activity requires identification of the product, which should be exclusively at the N′ (aminopropyl) end of spermidine, and the use of either a specific antiserum to SSAT, to show that all measured activity is lost when the SSAT protein is removed by immunoprecipitation (109), or one of the specific inhibitors described below. These considerations are less important when SSAT is induced and the non-SSAT reaction becomes a very small fraction of the total. The standard assay for SSAT in crude cell extracts involves using [14C]acetethyl-CoA and measuring the production of 14C-labeled polyamine product, which is readily separated from the substrate by virtue of its positive charge (84). A useful alternative, which eliminates the radioactivity, has been described recently using the highly fluorescent N1-dansylorspermine as a substrate (124). For kinetic assays using pure recombinant protein available in large amounts, a coupled assay measuring the release of CoA can be used (15, 61).

Specific antisera to SSAT that can be used for Western blots, assays, or immunohistochemical detection have been developed (22, 50, 109). A potent, specific, and cell-permeant inhibitor of SSAT is not yet available. Various multisubstrate analog inhibitors have been described (44, 61, 121), but their ability to enter cells and inactivate the enzyme in vivo has not been demonstrated.

**Localization of SSAT.** SSAT is generally considered to be predominantly cytosolic, but a recent study indicated a mitochondrial location for a large portion of the SSAT after induction by BE-3-3-3 in a breast cancer cell line (62). It has also been reported that SSAT is phosphorylated by casein kinase-1 and -2 and that this affects binding to mitochondrial membranes (17). Mitochondrial uptake therefore may be regulated by the phosphorylation state of SSAT, but the physiologically significance of these findings is unclear.

**Regulation of SSAT Content and Effects of Polyamines and Analogs**

SSAT regulation occurs at multiple levels, including transcription, mRNA processing, mRNA translation, and protein stabilization (Fig. 4A). Polyamines affect all of these steps. Transcription and translation are increased in the presence of high levels of polyamines or polyamine analogs, whereas degradation of the SSAT protein and incorrect splicing of the SSAT mRNA are reduced. This provides four separate reinforcing steps by which SSAT protein levels and activity are elevated when polyamine content is high.

**SSAT transcriptional regulation.** The Sat1 gene contains a polyamine-responsive element (PRE) with a sequence 5′-TATGACTAA-3′ that is located in a 31-bp stretch from −1522 to −1492 bp with respect to the SSAT transcriptional start site (135, 139) (Fig. 2B). This element allows the increased transcription of SSAT when polyamine content is high (Fig. 4B). A transcription factor, NF-E2-related factor 2 (Nrf-2), which is
known to regulate transcription of other genes via its interactions with members of the small Maf protein family, interacts constitutively with the PRE. Activation of SSAT transcription requires another protein, termed polyamine-modulated factor-1 (PMF-1), which partners with Nrf-2 and turns on the transcription of SSAT in response to polyamines. PMF-1 is a 165-amino acid protein with a predicted molecular mass of 120 kDa (137). PMF-1 pairs with Nrf-2 via a COOH-terminal coiled-coil region that interacts with the leucine-zipper region of Nrf-2. Mutations that alter the ability of these factors to interact destroy their ability to regulate the transcription of the Sat1 gene (136). It is not known how polyamines activate the PMF-1/Nrf-2 complex, and the presence of other essential proteins in the mechanism is not ruled out. Thioredoxin-1 reduces SSAT mRNA expression by decreasing PMF-1/Nrf-2 binding (67). This may contribute to the role of thioredoxin-1 in tumor development by increasing polyamine content.

Polyamine analogs such as BE-3-3-3 or BE-3-4-3 cause a huge increase in Sat1 gene expression in certain tumor cells. The initial mechanism of this superinduction is an increase in transcription due to the PRE. The Nrf-2 transcription factor appears to be present in high amounts in the nucleus only in the cell types that are capable of expressing high amounts of SSAT (135). Nrf-2 is a key regulator of genes that reduce toxic stresses and is itself regulated by Keap1-mediated degradation. Studies with drugs that increase Nrf-2 or mice with deletions of its gene have shown it plays a major role in protection against oxidative damage and environmental insults leading to tumor
development (150). The extent to which its ability to modulate polyamine metabolism via SSAT is also involved in this function of Nrf-2 is an important area for further investigation.

**SSAT transcript stability and processing.** Alternative splicing provides a second important regulatory step for the control of SSAT mRNA production (Fig. 4C). The SSAT pre-mRNA can undergo alternative splicing by the inclusion of an additional 100-bp exon that contains multiple premature termination codons between exons 3 and 4 (SSAT-X mRNA). This renders the SSAT-X variant a target for nonsense-mediated mRNA decay (70). Polyamines or analogs inhibit the exon inclusion and thus lead to more of the correct and stable mRNA. In addition to the loss of authentic SSAT mRNA by this incorrect splicing, translation of the SSAT-X mRNA would be expected to generate a truncated SSAT of only 71 amino acids. This protein might theoretically inactivate SSAT by forming heterodimers, providing another means by which SSAT activity would be low in cells with low polyamine levels. However, only one report (81) has described such a peptide, and this was found in nonphysiological conditions after transfection with a cDNA construct for the SSAT-X. The rapid loss of SSAT-X mRNA via nonsense-mediated mRNA decay may limit any accumulation in other cells, but SSAT-X mRNA has been found to accumulate in response to X-rays (99), viruses (104), and hypoxia (81). The induction by RNA viruses is easily explained, since the inhibition of host protein synthesis is known to stabilize transcripts targeted to nonsense-mediated mRNA decay.

Polyamines and analogs clearly increase the stability of SSAT mRNA (46–48, 89), and this also may contribute to the regulation. The half-life of SSAT mRNA was increased from 17 to 64 h in the presence of BE-3-4-3. The mechanism underlying this effect is unclear, and the use of actinomycin D to stop RNA synthesis over such a long period is questionable. SSAT mRNA content and half-life were also apparently increased by treatment with protein synthesis inhibitors (48, 90), and SSAT mRNA levels were increased by amino acid deprivation (5). The extent to which these effects may be due to the accumulation of SSAT-X mRNA is unclear.

**SSAT mRNA translation.** Translational regulation of SSAT synthesis has been reported by several groups (18, 105, 120). The polysomal distribution of SSAT mRNA in COS-7 cells transfected with a plasmid expressing the SSAT mRNA showed that most of the mRNA was present in fractions that sedimented more slowly than the monoribosome peak and was not being translated. After addition of a polyamine analog, BE-3-4-3, a significant proportion of the SSAT mRNA was moved into the small polysome region of the gradient consistent with translation (105). This translational regulation did not require either the 5' or 3'-UTRs of the mRNA, since constructs that lacked all of these regions gave similar results to constructs containing the entire mRNA sequence (105). These preliminary results consistent with translational regulation were recently confirmed and extended in studies in which FLAG-tagged SSAT or SSAT fused to luciferase was used (18). These studies used only the coding sequence of SSAT, confirming that regulation involved this sequence. Deletion analysis showed that two regions (localized to nucleotides 1–45 and 492–504) were involved in the stimulation of translation by polyamines. A cytoplasmic protein(s) that bound to the mRNA was detected using an electrophoretic mobility shift assay, and this binding was inhibited by BE-3-3-3 (18). These results are consistent with a model in which an inhibitory protein represses SSAT synthesis by binding to the mRNA, and polyamines and analogs increase SSAT synthesis by overcoming this repression (Fig. 4D). Isolation and characterization of the putative inhibitory protein would be of great interest; currently, it is not known whether this protein is specific for SSAT regulation or whether it could link polyamine content to the translation of other mRNAs.

**SSAT protein turnover.** The rapid turnover of SSAT protein was first demonstrated by showing that the activity in carbon tetrachloride-treated rats declined rapidly after treatment with cycloheximide (92). When a specific antibody became available, this observation was confirmed by measurement of the SSAT protein and a half-life of ~15 min was observed in thioacetamide-treated rats (109). The degradation of SSAT is carried out by the 26S proteasome after polyubiquitination (Fig. 4E) (36, 95). Rapid turnover of SSAT requires the Δ^156Δ164Δ171 sequence at the COOH-terminal end. Truncation to remove these residues, addition of two additional Lys residues to the COOH terminus, or mutation of the Glu acidic residues to Gln or Lys prevented degradation (36). The site of ubiquitin addition is not known for certain. There are multiple surface Lys residues in SSAT. Mutation of each individually to Arg did not block degradation, but the K87R mutant had a longer half-life than wild type or any of the other mutants (35), so Lys87 may be the preferred site, with other sites being used when this is altered.

Binding of polyamines or polyamine analogs to the SSAT protein greatly increases the half-life of SSAT to >12 h (36, 48, 96, 105). Such binding prevents the formation of polyubiquitinated SSAT (Fig. 4E) (15, 35, 97). This stabilization causes a large amplification of the effect provided by increased mRNA production and translation in response to the signal of polyamines or analogs.

**Induction of SSAT.**

Many factors, including polyamines, polyamine analogs, toxins, and stress pathways, cause large increases in SSAT activity and protein (reviewed in Refs. 7, 23, 135). Alterations of SSAT expression by nonsteroidal anti-inflammatory drugs (NSAIDs) have been studied in depth (see below), and changes in response to hormones and cytokines, including corticosteroids, estradiol, progesterone, calcitriol, growth hormone, parathyroid hormone, catecholamines, and IGF-1 are also well documented (Refs. 23, 42, 55, 116 and references therein). Ischemia-reperfusion injury has been shown to upregulate SSAT in brain (100, 151), kidney (148), and heart (58, 118). Natural products with important physiological/pharmacological effects, such as gossypol (27), 7β-hydroxycholesterol or 7β-hydroxyxysteroster (117), H2O2 (32), resveratrol (126), and interferons (66), also induce SSAT in target cells. The SSAT gene contains multiple sites for the binding of transcription factors that can respond to such stimuli (Fig. 2B). It is quite likely that many of these inducers may also elevate SSAT by raising the content of free polyamines, either by increasing polyamine synthesis or by releasing polyamines from bound sites. The mechanism(s) by which several classes of inducers alter SSAT content has been studied in depth as described below.

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Induction by polyamine analogs. All of the regulation sites respond to increased polyamine content in a way that increases SSAT levels and thus maintains polyamine homeostasis. The response to some polyamine analogs with NH₂-terminal alkyl groups is greater and more prolonged (1) due to a stronger effect on the regulation steps and 2) because the analogs are not substrates for SSAT, so their content does not decline in response to the induction of SSAT. The accumulation of SSAT protein that occurs in response to polyamine analogs clearly increases in vivo SSAT activity leading to excretion and degradation of cellular polyamines. However, it should be noted that binding of such polyamine analogs also inhibits the enzymatic activity, so the very high values for SSAT activity noted that binding of such polyamine analogs also inhibits the enzymatic activity, so the very high values for SSAT activity obtained by assays in vitro of extracts in which the analog is diluted may overestimate the in vivo activity.

The upregulation of the Sat1 gene transcription is critical for the cell-specific polyamine or analog-mediated increase in SSAT content, but, even in cell lines that show the largest induction in protein (200- to 1,000-fold), the increase in transcription is much less dramatic (46, 95, 135, 143). The signal is then amplified via the other mechanisms described above, with protein stabilization playing a major role. The strength of the additional increase in SSAT that occurs via translational and posttranslational mechanisms and the importance of such a high level of induction in response to polyamine analogs were also emphasized by studies in which constructs that express the SSAT mRNA were transfected into tumor cells (130). This gave a 4- to 10-fold increase in basal SSAT expression, but a much larger increase (180- to 3,500-fold increase) was seen when BE-3-3-3 was administered.

Induction by NSAIDs and resveratrol. NSAIDs increase SSAT activity by two mechanisms. One contributor to this effect is via NF-κB. Two NF-κB response elements occur in the SSAT gene (Fig. 2B) (31), and their occupation by NF-κB is increased by aspirin, leading to increased SSAT transcription (7). TNF-α also induces SSAT via NF-κB signaling (8). This provides a mechanism by which inflammatory stress increases SSAT and reduces polyamines. NSAIDs also produce an increased SSAT transcription mediated via the PPAR element and PPAR-γ (9). The SSAT gene contains two PPAR response elements (PPREs) (Fig. 2B). One of these, located at +48 bases relative to the transcription start site, is specifically bound by PPAR-γ and is clearly implicated in the induction by NSAIDs. The induction of SSAT by NSAIDs may be a factor in their ability to reduce carcinogenesis (6, 7). Similarly, resveratrol acts to increase SSAT by increasing transcription using the PPREs, and its ability to bring about this increase is blocked by a dominant negative of PPAR (126).

Conversely, expression of an activated Ki-ras gene reduces SSAT expression via this PPAR-γ-mediated system (52, 71). Transfection with a construct expressing Ki-ras decreased levels of PPAR-γ and SSAT activity. Increasing PPAR-γ by transient expression reversed the effects on SSAT and increased SSAT promoter activity. These effects may contribute to the carcinogenic effects of the ras oncogene, which increases polyamines by both elevating L-ornithine decarboxylase (ODC) and reducing SSAT.

Induction by chemotherapeutic drugs. Studies have shown SSAT induction in response to chemotherapeutic drugs such as platinum derivatives (59, 123) and 5-fluorouracil (4, 30), which are mediated via an increase in mRNA. This induction is greatly enhanced by simultaneous exposure to a polyamine analog, probably because this stimulates the additional translational and posttranslational steps shown in Fig. 4, D and E. The subsequent loss of polyamines may contribute to tumor cell death by apoptosis. The combination of polyamine analogs with these agents is therefore an attractive therapeutic strategy (60). Human ovarian carcinoma cell lines resistant to cisplatin and related drugs were found to show a much lower induction of SSAT in response to BE-3-4-3 than cisplatin-sensitive lines. This correlated with their reduced growth sensitivity to such analogs (89). The inability of the cisplatin-resistant cells to increase SSAT gene transcription was due to the presence of labile repressor proteins (90). Transient transfection with a plasmid expressing SSAT from a promoter not recognized by these repressors increased SSAT levels and restored sensitivity not only to BE-3-4-3 but also to cisplatin (91). These results are consistent with a role for SSAT in mediating the therapeutic effects of cisplatin, possibly by allowing more extensive interaction with DNA through the removal of DNA-bound polyamines.

Importance of SSAT induction in the therapeutic effects of polyamine analogs. A wide variety of polyamine analogs has been examined for use as cancer chemotherapeutic drugs, and some of the many promising results seen in laboratory experiments have been moved forward into clinical trials (14, 16, 24, 25, 56). Early studies with some analogs such as BE-3-3-3 showed that they produced a massive induction of SSAT and decline of polyamines in sensitive tumors, and it was postulated that their antitumor action was due to polyamine depletion mediated via both SSAT induction and repression of the polyamine biosynthetic enzymes. It is now clear that many polyamine analogs, which are also profoundly antiproliferative, do not act in this way and that there are multiple sites in cells on which polyamine analogs can exert therapeutic effects (16, 25, 65, 101, 133, 140).

However, for some analogs, SSAT does seem to be a critical target. Definitive proof of this was obtained in studies in which Chinese hamster ovary (CHO) cells resistant to BE-3-3-3 and BE-3-4-3 were derived via repeated exposure to increasing levels of BE-3-4-3. It was found that these resistant cells did not induce SSAT in response to the analogs and that expression of SSAT through transfection of the human cDNA restored this sensitivity (96). The mutant CHO cells were found to contain a point mutation in the SSAT gene that alters the coding sequence, changing Leu156 to Phe. This mutation not only greatly impairs the activity but also prevents BE-3-3-3 from protecting the mutant protein from polyubiquitination and degradation (97). Subsequent determination of the crystal structure of SSAT explains these results, since the L156F mutation would change the binding surface for the aliphatic carbons of the substrate/inducer at positions 8–10 (15). These results once again show the importance of the SSAT protein-stabilizing effect in the increase in SSAT levels in response to polyamine analogs, since there was no difference in mRNA level between the wild-type and the resistant line.

Transfection of a construct expressing high levels of SSAT also renders tumor cells much more sensitive to killing by BE-3-3-3 (130). Surprisingly, however, embryonic stem cells from SSAT knockout mice were actually more sensitive to BE-3-3-3 than controls, even though polyamine pools were unchanged by the analog and the survival of SSAT knockout
mice treated with high doses of BE-3-3-3 (125 mg/kg) was much poorer than wild-type mice (102). One possible explanation for this is that there may be multiple cell targets for BE-3-3-3, and it is possible that in some cells, the binding of the analog to SSAT can protect from interaction with other sites.

Induction of SSAT may have an antitumor effect not only by reducing polyamine content. As described below, high levels of SSAT cause a fall in acetyl-CoA and malonyl-CoA, which would decrease fatty acid synthesis. Drugs inhibiting acetyl-CoA carboxylase that alter fatty acid synthesis have an antitumor effect (13). Also, stimulation of the SSAT/APAO pathway by polyamine analogs may kill tumor cells via an increase in cellular oxidative stress (76, 112, 135). This effect may be enhanced by the induction of spermine oxidase (SMO) by these analogs (129, 135, 138); unlike APAO, SMO is not peroxisosomal and may therefore produce more widespread cellular damage. BE-3-3-3 had a variety of downstream effects in brain tumor cells leading to cell death, notably by targeting mTOR (mammalian target of rapamycin)-mediated protein synthesis (76).

Transgenic Manipulation of SSAT Levels

**Inactivation of the Sat1 gene.** Resting SSAT levels are very low, and it is perhaps not surprising that knockout of the Sat1 gene had little effect on polyamine content with only a slight increase in the spermidine-to-spermine ratio in either embryonic stem cells (103) or mouse tissues (102). The need for SSAT in the back conversion of spermine and spermidine into putrescine was confirmed in these cells; the small conversion of spermine into spermidine in the absence of SSAT is probably due to the direct oxidation of spermine by SMO (129, 135, 138) (Fig. 1).

**Transgenic increase of SSAT.** Multiple lines of transgenic mice and transgenic rats overexpressing SSAT have been described (reviewed in Refs. 73, 74, 107). These include mice with an increased Sat1 gene copy number in which the inserted genes presumably respond to physiological stimuli, rats and mice in which a Sat1 sequence is placed under the control of a metallothionein promoter, and mice in which a sequence encoding SSAT was expressed from a keratin-6 (K6) promoter to target expression to the skin. Widespread transgenic expression of SSAT using its own promoter or the metallothionein promoter led to a substantial reduction in spermidine/spermine pools with a large increase in putrescine and in N1-acetylspermidine and excretion of acetylated polyamines, including N1,N2-diacylspermine (73). These changes are consistent with the role of SSAT in regulating polyamine levels as described above. These mice also showed a wide variety of other defects, including hair loss, female infertility, weight loss, CNS effects, and altered lipid metabolism, and a tendency to develop pancreatitis (see Fig. 5).

**Physiological or Pathophysiological Effects of Altered SSAT Expression**

SSAT has been implicated in multiple disease states (Fig. 5). These include death of pancreatic cells, blockage of regenerative tissue growth, obesity and diabetes, keratosis follicularis spinulosa decalvans, behavioral changes affecting a tendency to suicide, cell migration, gene regulation, and carcinogenesis. Some of these changes may result from direct interaction of SSAT with other proteins, and TLAT may also influence gene regulation by protein binding (Fig. 5). More details of these changes and their causation by altered SSAT levels are given below.

**Pancreatitis.** Transgenic rats with overexpression of SSAT from the metallothionein promoter provide an interesting model for pancreatitis (2, 69). The pancreas has a very high spermidine content and is very sensitive to reduction in the level of spermidine and spermine. Increased polyamine catabolism resulting from a very high level of SSAT induction following treatment of the transgenic rats with zinc led to acute necrotizing pancreatitis. This effect is clearly due to polyamine depletion, since replacement of the natural polyamines with methylated polyamine analogs that are not substrates for SSAT prevented the disease (69, 98, 114). It appears that loss of higher polyamines from cellular structures leads to premature zymogen activation and autodigestion of acinar cells. Pancreatic damage occurs very rapidly after SSAT induction in these transgenic animals with an increase in cathepsin B release within 2 h after induction of SSAT. It is also possible that inflammatory and oxidative stress mediators formed via the SSAT/APAO pathway may be involved, since serum inflammatory cytokine levels and NF-κB were activated (98). Although this transgenic rat system is simply an experimental model, it has been suggested with some supporting evidence that loss of polyamines via elevated polyamine catabolism may be a more general mechanism leading to pancreatitis (69, 115). If this is the case, the treatment with methylated polyamine analogs may have therapeutic value.

**Ischemia-reperfusion injury.** The loss of polyamines may also contribute to organ damage after ischemia followed by reperfusion. Such treatment has been shown to increase SSAT expression in kidney, heart, and brain (58, 100, 148). Increased SSAT levels in kidney epithelial cells gave rise to DNA damage and activated cell cycle check points leading to G2 arrest (147). However, in ischemic myocardium, SSAT was

![Fig. 5. Summary of effects of altered SSAT expression. As described in the text, the factor(s) linking altered SSAT activity and the phenotype indicated is not fully understood in some cases, but the diagram provides speculative information as to the most likely causes. KFSD, keratosis follicularis spinulosa decalvans; HIF-1α, hypoxia-inducible factor-1α; α9β1, integrin.](http://ajpendo.physiology.org/10.1152/ajpendo.00912.2007)

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upregulated via 5′-AMP-activated protein kinase (AMPK) in response to ATP depletion and appeared to act protectively to prevent cell death (118).

Liver regeneration. It has been known for many years that partial hepatectomy leading to liver regeneration is accompanied by a very rapid and massive increase in polyamine biosynthesis (113). More recent studies have confirmed that the increase in spermidine is essential for DNA synthesis and the increase in hepatocyte proliferation (3, 68). Transgenic overexpression of SSAT prevents liver regeneration unless 1-methylsperrmidine (which is not a SSAT substrate) is provided (68, 115). Since SSAT overexpression further increases putrescine at the expense of spermidine, these studies show clearly that spermidine is the critical polyamine needed for liver regeneration, even though putrescine is increased more rapidly and to a greater extent by partial hepatectomy.

Hair loss. The loss of hair in SSAT transgenic mice appears to be due to the deleterious effects of very high levels of putrescine on hair follicle development. Mice that overexpress ODC also are hairless (122), and double transgenic mice with increased SSAT and ODC have even more severe skin changes (110). K6-SSAT transgenic mice in which there was a very modest increase in SSAT and putrescine in the skin showed little alteration of the hair cycle but had a slight hair loss after repeated treatment with 12-0-tetradecanoylphorbol-13-acetate (TPA), which activates the K6 promoter (37).

Effects on carbohydrate and lipid metabolism. Preliminary studies with the mice that have the Sat1 gene inactivated showed no major metabolic deficiencies into early adult life and a normal life span, although at later ages there was a development of insulin resistance (102). More detailed studies have indicated that the lack of SSAT causes an accumulation of body fat with an increase in white adipose tissue and glucose oxidation (75). When placed on a high-fat diet, the Sat1 gene-knockout mice gained much more weight and lipid compared with the wild-type mice. These results are consistent with the effects of general overexpression of SSAT, which had the opposite effect as described below. Early characterization of mice overexpressing SSAT due to an increase in the Sat1 gene copy number showed that these mice not only had the expected alterations in polyamine content but also had profound changes in lipid metabolism (reviewed in Refs. 73, 74). Later studies confirmed that there was a decrease in white adipose tissue and a loss of body fat with high basal metabolic rate, improved glucose tolerance, and high insulin sensitivity (75, 111).

Porter and colleagues (75, 82) have proposed that the high levels of SSAT increase metabolic flux through the polyamine pathway, because ODC (and possibly other biosynthetic enzymes) is increased in response to the reduction in polyamines caused by SSAT. This sets up a cycle in which acetyl-CoA is depleted (Fig. 6). The fall in acetyl-CoA and the related fall in malonyl-CoA, removing its inhibition of fatty acid oxidation, produce a large increase in glucose and palmitate oxidation and a loss of fat (Fig. 6B).

Direct evidence for the increase in polyamine flux was observed in elegant studies using 4-fluoro-ornithine (80, 82). This is converted to the corresponding fluoropolyamines, which can be separated by HPLC from normal polyamines. The rate of fluorolabeling of the polyamine pool provides a means of measuring flux. When SSAT was increased using a tetracycline-regulated construct transfected into LNCaP prostate carcinoma cells (80), the spermidine and spermine pools were maintained by a compensatory increase in ODC and S-adenosylmethionine decarboxylase (AdoMetDC) activities, despite a large increase in acetylated polyamines (80, 82). Increasing SSAT 50-fold led to a 6- to 20-fold increase in the biosynthetic enzyme activities and a >5-fold increase in the rate of labeling of the polyamine pool. Excretion of the acetylated polyamines rather than oxidation via APAO (Fig. 6A) was the primary reason for the increased flux, since it was only slightly decreased when the APAO inhibitor MDL-72527 was present.

The cycle shown in Fig. 6A also leads to a significant loss of ATP, since all three phosphate bonds in ATP are broken when methionine is converted into AdoMet and two molecules of AdoMet are needed to form spermine. The synthesis of spermidine and spermine converts AdoMet to 5′-methylthioadenosine, which is salvaged back to methionine and adenosine via a series of reactions (106). The cycle thus uses up ATP and

![Fig. 6. Effect of SSAT-induced futile cycling of polyamine metabolism on lipid metabolism. A: depletion of ATP and acetyl-CoA and increased oxidative stress resulting from SSAT activity. B: alteration of adipose tissue fatty acids by SSAT-induced polyamine flux.](Image)
acetyl-CoA, forming adenosine, N-acetylaminopropanal (which is reduced to β-alanine), H₂O₂, and CO₂. It is probable that the very significant loss of ATP associated with this SSAT-driven futile cycle at high SSAT levels (Fig. 6) also contributes to the effects on lipid and carbohydrate metabolism as suggested by Pirinen et al. (111). There was an increase in AMPK, which led to PPAR-γ coactivator 1α (PGC-1α) activation, in white adipose tissue cells in SSAT-overexpressing transgenic mice when the cells were treated with BE-3-3-3 to further increase SSAT production (111). The increase in PGC-1α leads to the enhanced expression of the OXPHOS genes in the transgenic mice, causing a reduced fat mass and improved glucose tolerance. The decrease in ATP and the levels of AMPK and PGC-1α were restored toward normal by exposing the mice to α-difluoromethylornithine (DFMO) to inhibit ODC. This reduces the flux through the futile cycle. Such DFMO treatment reversed the decrease in white adipose tissue and body fat in the SSAT transgenic mice (111). These studies suggest that drugs enhancing polyamine catabolism particularly by induction of SSAT may be useful in the treatment of obesity and type 2 diabetes.

**Effects of human genetic alterations in SSAT. Suicidal tendencies.** Low SSAT gene expression has been linked to a propensity to suicide (119). This study using microarray analysis was supported by studies with reverse transcription-polymerase chain reaction, immunohistochemistry, and Western blotting for SSAT. It was found that the SSAT content was lower in the brains of persons attempting and committing suicide than in normal controls. This was due to reduced levels of SSAT mRNA that correlated with the C allele of the Sat1 342A/C polymorphism, which is located in the vicinity of the PRE region of the Sat1 gene promoter (119). On the basis of this detailed study and the strong induction of SSAT by treatment with BE-3-3-3, it has been suggested that BE-3-3-3 might have a therapeutic benefit in psychiatric patients with a high risk of suicide (43). However, it is unknown to what extent BE-3-3-3 can induce SSAT in the brain. Although brain was not examined, the increase in SSAT in normal tissues was much less than in tumor tissues of patients treated with BE-3-3-3 when examined using immunohistochemistry (50, 51).

The mechanism by which SSAT is linked to behavioral effects is unknown. There has been very little study of SSAT in the brain, but there is good evidence that polyamines influence the activity of ion channels, including N-methyl-D-aspartate receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropanic acid receptors, K⁺ channels, and Ca²⁺ channels. Also, an inherited defect in spermine synthase causes Snyder-Robinson syndrome, an X-linked mental retardation condition (26). SSAT-induced alterations in polyamine content could therefore alter brain function. Kainate-induced seizures increase SSAT activity (72), and transgenic overexpression of SSAT protects from kainate (77) and epilepsy-like seizure activity induction by pentylenetetrazol (77). These SSAT transgenic mice also showed a reduced activity and spatial learning impairment (78).

**Keratin 10 (KRT10) overexpression may be linked to the rare X-linked disease keratosis follicularis spinulosa decalvans (KFSD).** A patient with this disease was found to have a gene duplication that included the Sat1 region of the X chromosome that encodes SSAT. Cultured fibroblasts from the patient had a threefold increase in SSAT, a reduction in spermidine, and a twofold increase in putrescine. KFSD causes follicular hyperkeratosis and other alterations that affect vision. The skin changes are roughly similar to those seen in SSAT-overexpressing mice. It is possible that gain-of-function mutants in the Sat1 gene are a general cause of KFSD (54).

**Polymorphisms in SSAT.** There has been surprisingly little investigation of human variants in SSAT. In addition to alterations in the promoter region, several alterations in the coding region of the gene have been described including K26E, M30R, L89F, F103L, and R121S, but it is not yet known how these alterations might affect SSAT activity and stability.

**Interaction of SSAT with integrin signaling.** SSAT has a specific and functional interaction with the α9β1-integrin (28). This integrin, which mediates cell migration, is expressed on migrating cells such as leukocytes and binds to multiple ligands that are present at sites of tissue injury and inflammation. SSAT (via its NH₂-terminal region) binds to the α9 cytoplasmic domain, and this interaction is needed for α9β1-mediated migration. Reduction of SSAT expression using small interference RNA inhibited α9-dependent cell migration in CHO cells, and overexpression of wild-type SSAT increased migration. This effect appears to require SSAT catalytic activity, since the R101A/E152K mutant was ineffective (28). Although the possibility that SSAT interaction brings about its effects by acetylating as-yet-unidentified substrates (which could possibly be proteins) is not totally ruled out, evidence was obtained recently that it may act through localized catabolism of spermidine and/or spermine (40). These polyamines block K⁺ efflux through inward rectifier potassium (Kir) channels (83). It was found that knockdown of a single subunit, Kir4.2, or treatment with barium, a Kir channel inhibitor, specifically inhibited α9-dependent cell migration. Also, α9β1 and Kir4.2 colocalized in focal adhesions at the leading edge of migrating cells. These results strongly support a novel pathway through which the α9-integrin subunit stimulates cell migration by localized polyamine catabolism and modulation of Kir channel function.

It should be noted that it is likely that the α9-integrin may have additional effects that are not SSAT dependent. The phenotype of SSAT knockout mice and α knock out mice are very different. Mice homozygous for a null mutation in the α-subunit gene accumulate large volumes of pleural fluid after birth and develop respiratory failure and die between 6 and 12 days of age. It appears that α9-integrin is required for the normal development of the lymphatic system, including the thoracic ducts (64). In other studies, α9 has been shown to interact with vascular endothelial growth factors and to contribute to VEGF-induced angiogenesis and lymphangiogenesis (127).

**Gene regulation by SSAT and TLAT.** Gene regulation via hypoxia-inducible factor 1. Hypoxia-inducible factor 1 (HIF-1) is a multisubunit protein that regulates transcription at hypoxia response elements (149). SSAT binds to the HIF-1α subunit and promotes its ubiquitination and degradation (11). TLAT, which has similar overall structure to SSAT (see Fig. 3), also binds to HIF-1α and brings about this ubiquitination and degradation (10). However, the effects of TLAT occur via stabilizing the complex of hydroxylated HIF-1α, which is formed under aerobic conditions, with von Hippel-Lindau protein (VHL) and elongin C, whereas SSAT does so by stabilizing the interaction of the form of HIF-1α that is present...
in hypoxic conditions with RACK-1 and elongin C. Thus SSAT and TLAT promote the O2-independent and O2-dependent degradation of HIF-1α, respectively. It is probable that the effect of SSAT requires its acetyltransferase activity, since the inactive R101K mutant was ineffective (11), but no direct evidence of acetylation of any of the proteins involved or localized changes in polyamines is available. Mutation of Ser82 and Thr83 in TLAT, giving the inactive S82D/T83A mutant (87), reduced its effect on HIF-1 binding of TLAT influence degradation. Although this is highly speculative, it is interesting to note that TLAT can act on 5-hydroxylysinuric, which might be formed in the target HIF-1α by a lysine hydroxylase using O2. As described above, SSAT can acetylate an unmodified Lys in itself (15). Therefore, it may be able to target the same Lys in HIF-1α as TLAT without the need for oxidative modification. The effects of SSAT and TLAT on HIF-1α may have a variety of downstream effects. This transcription factor regulates transcription of hypoxia-inducible genes involved in angiogenesis, glucose transport and metabolism, erythropoiesis, inflammation, apoptosis, and cellular stress (149).

**GENE REGULATION VIA NF-κB.** TLAT has also been reported to bind to the p65 subunit of NF-κB (128). This binding increased the ability of p65 to stimulate transcription of a reporter gene with an NF-κB binding site. It was also shown that TLAT was present in the promoter complex of the NF-κB-regulated genes after stimulation by TNF-α. TLAT led to an increase in TNF-α-induced reporter gene expression and cooperated with the CREB-binding protein and p/CAF coactivators in increasing the response to TNF-α (128). Thus TLAT may cooperate with the NF-κB transcription factor in signaling to regulate genes involved in response to infection and inflammation and in cell development.

**Effects of SSAT on Carcinogenesis**

The pleiotropic effects of the changes associated with widespread high levels of SSAT expression in transgenic mice with increased Sat1 gene copies complicate the use of these animals for specific studies in carcinogenesis, but such studies have been carried out with these mice and with the K6-SSAT transgenic mice, which had only modest alterations in polyamines limited to the skin and do not suffer from this drawback. Since elevated levels of polyamines are clearly associated with neoplastic growth, it might be expected that an increase in SSAT and a subsequent reduction in polyamines would impair the development of neoplasia. This was indeed found in a study in which SSAT-transgenic mice on the C57BL/6J background bred with TRAMP mice, which develop prostate tumors due to an expression of SV40 T antigens from the probasin promoter, showed a reduction in tumor incidence (79). Also, in earlier studies using mice on a hybrid CD2F1 background, it was reported that these mice, which have a ubiquitous SSAT overexpression due to an increase in the Sat1 gene copy number, had decreased tumor incidence in the skin in response to a two-stage tumorigenesis protocol with initiation by 7,12-dimethylbenz[a]anthracene (DMBA) and promotion with TPA (73, 110).

However, other studies with the same SSAT transgenic mice on the inbred C57BL/6J background showed that when they were bred with Apcmin+ mice, which are predisposed to intestinal tumor formation, the incidence of intestinal tumors was increased (125). Conversely, crosses of the Apcmin+ mice with SSAT knockout mice led to a 75% reduction in tumor burden (125). Furthermore, K6/SSAT C57BL/6J transgenic mice in which a SSAT cDNA was driven by the K6 promoter to express only in the skin showed a striking increase in tumor incidence and development in response to the two-stage tumorigenesis protocol with DMBA and TPA (37). There was an increase in both the number and size of skin tumors and in their progression to carcinomas in these mice. This effect is particularly remarkable, since there was only a very small overexpression of SSAT in the resting state. SSAT activity was increased further in the tumors. This may be due to increased expression from the K6 promoter, which responds to increased cell proliferation, reinforced by stabilization of the SSAT protein by increased polyamines and possibly by other factors. Polyamine acetylation was significantly increased in the tumors, but it is unlikely that the acetylpolyamines themselves are the major factor, since the increased tumor incidence was reduced by treatment with MDL-72527, an inhibitor of APAO, which prevented degradation of the acetylated polyamines and further increased their content (134). This focuses attention on the products of the SSAT/PAAO pathway, which include putrescine, reactive oxygen species, and N-acetyliminopropional. It is certainly possible that oxidative stress resulting from SSAT/PAAO may contribute to the increased tumor development, but several experiments suggested that putrescine may be a critical factor. Blocking ODC activity to reduce de novo putrescine production with the ODC inhibitor DFMO resulted in the complete regression of established tumors, and breeding of the K6-SSAT mice with K6-AZ mice (which express antizyme, a negative regulator of ODC) also blocked the development of tumors (134).

Thus the increased susceptibility to carcinogenic stimuli in mice overexpressing SSAT may be due to a compensatory increase in the biosynthesis of polyamines, as suggested by Tucker et al. (125). In the K6-SSAT skin and the intestinal tumor models (37, 125), transgenic SSAT expression led to increased tumor development and produced a large increase in putrescine but only a small decline in spermidine and spermine. This is consistent with a general upregulation of polyamine biosynthesis to replace those polyamines degraded in the SSAT/PAAO pathway (see Fig. 6). In the prostate model and the skin with more general unregulated SSAT overexpression (79, 110), this upregulation may not occur to an extent able to maintain polyamine levels. In those cell types where it takes place, the compensatory upregulation of polyamine biosynthesis to replace those polyamines degraded via the SSAT/PAAO pathway may result in an increased level of putrescine via synthesis as well as that arising from an increased degradation of the higher polyamines. It is interesting that these results focus attention on putrescine rather than spermidine as the critical polyamine in promoting neoplastic growth. Although putrescine is often considered only as a precursor of the polyamines spermidine and spermine, it may influence gene regulation either by its own actions or by modulating the effects of these amines.
SSAT-MEDIATED METABOLIC REGULATION

Review

Summary and Future Directions

The importance of SSAT is emphasized by the intricate regulation of SSAT protein levels. Multiple effects at all of the steps at which SSAT regulation can occur ensure close control of the cellular polyamine content, which, in turn, may influence cell growth and ion channel activity. Although the mechanisms controlling SSAT levels are understood in broad outline, many details need to be clarified, such as, for example, the means by which the PRE is linked to transcription by polyamines and the detailed characterization of the proteins involved in the inhibition of SSAT mRNA translation. A more detailed understanding of these mechanisms may allow new pharmacological and physiological agents altering SSAT synthesis to be identified.

It is now clear that SSAT is not only a key regulatory enzyme in polyamine metabolism but also links the polyamine pathway to other parts of cell physiology areas in several ways, including alteration of acetyl-CoA and ATP levels and production of oxidative stress. The polyamine biosynthetic enzymes ODC and AdoMetDC are upregulated in response to a decrease in polyamines. Therefore, an elevation of SSAT increases polyamine flux in what is essentially a futile cycle. In nonhepatic tissues, polyamine biosynthesis is a major consumer of AdoMet, and this consumption is increased by the SSAT-driven polyamine cycle. Further experimentation is needed to determine the extent to which the alterations in ATP and acetyl-CoA levels that stimulation of this cycle brings about can affect not only lipid and carbohydrate metabolism but also other cellular processes.

Additional effects of SSAT and the related protein TLAT occur via interaction of these proteins with integrins and other binding partners for these acetyltransferases may well be discovered. These interactions could bring about changes in the function via steric alterations of the target protein upon binding, local acetylation of low-molecular-weight substrates such as polyamines, or protein acetylation. At present, it is not possible to be sure which of these is the key mechanism. Effects of TLAT are not likely to involve polyamines but, with the exception of the self-acetylation of SSAT, the ability of either protein to acetylate proteins has not been demonstrated clearly. However, the inactivity of mutants at the catalytic site of SSAT or TLAT to alter αβ1-integrin or HIF-1α function provides strong evidence that the effects are not simply due to protein-protein interactions.

A major problem in the polyamine field is that there is no available method to measure local polyamine concentrations or even the free polyamine concentration in the cell. A substantial fraction of the strongly positively charged polyamines is likely to be bound to cellular components such as DNA, ribosomes, and membranes. The free concentration may be critical for modulation of cellular functions, and this could be the key target of SSAT activity, which produces both general and local reductions in polyamine content by acting on the free pool. SSAT is also likely to alter the balance between free and bound polyamines by reducing the net positive charge via acetylation.

The concept that polyamine metabolism may be a good target for the design of agents for cancer treatment and chemoprevention is well established (12, 25, 52, 53, 88). Excellent inhibitors of the biosynthetic enzymes such as DFMO are currently available, and these have been used successfully for the treatment of diseases caused by parasites. Unfortunately, these inhibitors have not proven useful for cancer therapy, since the compensatory increase in the target enzymes and polyamine uptake in response to a drop in polyamine content overrides their antiproliferative effects. Induction of SSAT by polyamine analogs provides an alternative approach that is undergoing trials, and combination therapy may be valuable (12, 16, 25, 133). Although it has been suggested that SSAT may be a useful target for many other diseases based on the plethora of effects of SSAT described in this review, it is obvious that such therapeutic intervention must be very carefully designed to avoid unwanted side effects due to alterations in nontarget cells. Unless a means to selectively alter SSAT in the key cell type is perfected, it seems unlikely that these approaches will be successful. Similarly, although DFMO is an excellent candidate drug for cancer chemoprevention (12, 52, 53), the complexity of the effects of SSAT induction in carcinogenesis and the multiple effects of increasing SSAT do not support the use of SSAT inducers for such purposes without extensive experimentation and safety testing.

ACKNOWLEDGMENTS

I am very grateful to Dr. Maria Bewley for preparing Fig. 2, to Dr. Robert A. Casero, Jr., for helping with Fig. 3, to Drs. Diane E. McCloskey, David J. Feith, and Lisa. M. Shantz for helpful discussions and suggestions on the manuscript, and to all former and current laboratory members who worked on SSAT. I apologize that some references to excellent papers on SSAT may have been omitted due to space considerations.

GRANTS

Research on polyamines in my laboratory is supported by National Institutes of Health Grants CA-018138 and GM-26290.

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