Chapter 5
Mammalian Polyamine Catabolism

Tracy Murray-Stewart and Robert A. Casero Jr.

Abstract Intracellular mammalian polyamine catabolism occurs through two distinct pathways, both of which culminate in oxidation reactions that generate highly reactive, potentially toxic by-products. In the back-conversion to spermidine, spermine can either undergo direct oxidation by spermine oxidase (SMOX) or be acetylated by spermidine/spermine N^1-acetyltransferase (SSAT), followed by subsequent oxidation by acetylpolyamine oxidase (APAO). Spermidine undergoes acetylation and oxidation back to putrescine through this same SSAT/APAO pathway. Polyamines are absolutely essential for cell viability and proliferation, and polyamine biosynthesis and intracellular concentrations are frequently upregulated in hyperproliferative conditions such as cancer. As a result, many studies have successfully focused on the induction of polyamine catabolism as a rational target for antiproliferative chemotherapeutic intervention. However, it is also becoming apparent that chronically elevated levels of polyamine catabolism in nontumorigenic cells can have disease implications. A variety of stimuli, including microbial pathogens, inflammatory signals, and tissue injury, have now been identified to induce the polyamine catabolic enzymes. In addition to the back-conversion of polyamines, these reactions also release the reactive oxygen species precursor hydrogen peroxide as well as potentially toxic aldehydes. These metabolites as well as the reduction in spermine and spermidine levels can have deleterious physiological effects resulting in the manifestation and promotion of multiple pathologies. This chapter focuses on recent discoveries in the regulation of the mammalian polyamine catabolic enzymes and the pathophysiological effects of this upregulation.

Keywords Aldehyde • Epigenetic • Inflammation • Reactive oxygen species • Spermidine/spermine N^1-acetyltransferase • Spermine oxidase

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5.1 Introduction

Putrescine, spermidine, and spermine constitute the naturally occurring mammalian polyamines. As in all cells, the mammalian polyamines are absolutely essential for viability through their contributions to critical cellular functions, including nucleic acid and protein synthesis, transcriptional and translational regulation, and macromolecular structural integrity (Pegg 1988, 2009; Saini et al. 2009; Park et al. 2010). Spermine, in particular, has also been shown to provide significant protection against oxidative damage (Ha et al. 1998a, b; Rider et al. 2007). For these functional interactions, polyamine homeostasis must be tightly regulated: an excess of intracellular polyamines becomes rapidly toxic (Tabor and Rosenthal 1956), whereas highly upregulated polyamine catabolism reduces the natural intracellular polyamines and generates toxic by-products (Wang and Casero 2006).

5.2 The Mammalian Polyamine Catabolic Enzymes and Their Metabolites

In mammalian cells, the catabolism of spermine to spermidine occurs via one of two distinct pathways. As a substrate for spermidine/spermine N^1-acetyltransferase (SSAT), spermine can be converted to N^1-acetylspermine, which is subsequently oxidized by the FAD-dependent acetylpolyamine oxidase (APAO) to form spermidine. Conversely, spermine can be directly oxidized by spermine oxidase (SMOX) to form spermidine. Spermidine is then back-converted to putrescine through the two-step SSAT/APAO reaction that includes an N^1-acetylspermidine intermediate (Fig. 5.1).

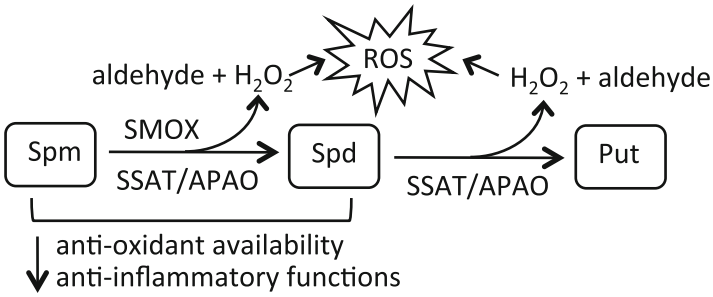

Fig. 5.1 The mammalian polyamine catabolic pathway. Spermine (*Spm*) is back-converted to spermidine (*Spd*) by either spermine oxidase (*SMOX*) or spermidine/spermine N^1-acetyltransferase (*SSAT*) followed by acetylpolyamine oxidase (*APAO*). Spermidine is further back-converted to putrescine (*Put*) through the same SSAT/APAO mechanism. Both oxidation reactions generate the reactive oxygen species (*ROS*) precursor H_2O_2 and aldehydes as by-products. The resulting reduction in spermine and spermidine pools implies diminished antioxidant and antiinflammatory functions

5.2.1 *Spermidine/Spermine N^1-Acetyltransferase and N^1-Acetylpolyamine Oxidase*

SSAT is the rate-limiting enzyme of the polyamine catabolic pathway that catalyzes the transfer of an acetyl group from acetyl coenzyme A to the N^1 position of spermine or spermidine (Casero and Pegg 1993; Pegg 2008; Matsui et al. 1981). The resulting molecule has a reduced positive charge that alters its binding affinity for cellular macromolecules and facilitates its export from the cell. Additionally, N^1-acetylated spermine or spermidine can be oxidized by APAO, resulting in spermidine or putrescine, respectively, hydrogen peroxide (H_2O_2), and 3-acetamidopropanal (Holtta 1977; Wu et al. 2003; Wang et al. 2005b). H_2O_2 is a potential reactive oxygen species (ROS); however, in most cases, the peroxisomal localization of the APAO enzyme appears to protect the cell from its oxidative effects.

5.2.2 *Spermine Oxidase*

The *SMOX* gene encoding spermine oxidase is alternatively spliced, and multiple isoforms have been characterized in both human and mouse (Wang et al. 2001; Murray-Stewart et al. 2002, 2008; Cervelli et al. 2003). The catalytically active SMOX isoforms are FAD-dependent enzymes that directly oxidize spermine to yield spermidine, H_2O_2, and the aldehyde 3-aminopropanal. Importantly, these isoforms are found in significant amounts in both the cytoplasm and nucleus, resulting in the production of H_2O_2 as an ROS precursor in close proximity to DNA and chromatin while catalyzing the oxidation of the free-radical scavenger spermine (Murray-Stewart et al. 2008; Cervelli et al. 2004; Bianchi et al. 2005). SMOX activity therefore has the potential to significantly contribute to cellular oxidative damage and subsequent disease development. Additionally, the 3-aminopropanal produced through SMOX activity can be further metabolized to form acrolein, which also has toxic cellular effects and physiological implications.

5.3 Induction of Polyamine Catabolism

Regulation of SSAT occurs at nearly every level from transcription through protein stabilization, resulting in an enzyme that is highly inducible (Casero and Pegg 1993; Pegg 2008). Elevated levels of the natural polyamines themselves stimulate SSAT induction, and polyamine analogues have been extensively studied for their ability to highly induce SSAT activity in tumor cells as a chemotherapeutic strategy (Nowotarski et al. 2013; Casero and Marton 2007). Other stimuli for SSAT induction include certain proinflammatory cytokines, nonsteroidal antiinflammatory drugs (NSAIDs), hormones, stress, and common cytotoxic drugs such as cisplatin (Casero and Pegg 2009). As the acetylated polyamine substrates for oxidation by

APAO are produced only through SSAT activity, APAO is in general constitutively expressed and rate limited by SSAT.

In contrast to APAO, oxidation of spermine via SMOX is inducible; however, most of its regulation appears to be at the level of transcription (Wang et al. 2005a). Similar to SSAT, SMOX can be induced by polyamine analogues and certain pro-inflammatory cytokines, leading to oxidative DNA damage. SMOX is also induced upon microbial pathogen infection, in states of chronic inflammation, and following tissue injury (Casero and Pegg 2009). Recent advances in the regulation and induction of the polyamine catabolic enzymes and their pathophysiological implications are discussed in the text that follows.

5.3.1 Epigenetic Regulation of Mammalian Polyamine Catabolism

Epigenetics refers to heritable alterations in gene expression that are not the result of changes in nucleotide sequence. Mechanisms that induce these changes include posttranslational modifications of histone proteins, such as acetylation, methylation, and phosphorylation, and the methylation of CpG dinucleotides. Few studies have focused on the direct epigenetic regulation of the polyamine catabolic enzymes, although the promoter regions of both *SAT1* and *SMOX* genes contain identifiable CpG islands, suggesting the potential for transcriptional regulation through DNA methylation. The influence of methylation on SSAT expression was initially identified in lung cancer cell lines derived from female patients. The *SAT1* gene is located on the X chromosome; therefore, the female cell lines expressed variable levels of basal SSAT expression and responsiveness to polyamine analogues that correlated with the expression of one or both alleles, and this expression was regulated by DNA methylation (Mank-Seymour et al. 1998). Recent studies in the prefrontal cortex have also correlated *SAT1* promoter DNA CpG hypermethylation with decreased SSAT mRNA expression (Fiori and Turecki 2011). As DNA methylation is often increased in cancer, the downregulation of polyamine catabolic enzymes via DNA hypermethylation could provide a mechanism for maintaining the high levels of polyamines required for tumor proliferation.

SSAT expression is also indirectly regulated through epigenetic mechanisms. The *SAT1* gene promoter contains a polyamine-responsive element (PRE) that enables transcriptional activation via the binding of NRF2 and polyamine-modulating factor 1 (PMF1) (Wang et al. 1998, 1999). In human non-small cell lung cancer cell lines that respond to polyamine analogue treatment with a large induction of SSAT, NRF2 is constitutively bound at the PRE by alterations of the KEAP1 protein, which normally sequesters it in the cytoplasm (Singh et al. 2006; Itoh et al. 1999). Recent studies have shown that NRF2-dependent transcriptional regulation of SSAT is influenced by the histone acetylation status of a specific microRNA, miR-200a, which targets the 3′-UTR of the KEAP1 mRNA. Treatment of polyamine analogue-resistant small cell lung cancer cells with a histone deacetylase (HDAC) inhibitor increased expression of miR-200a, which subsequently down-regulated KEAP1 mRNA and protein and allowed the translocation of NRF2 to the

nucleus. NRF2 occupancy was enriched at the PRE of the SSAT promoter, resulting in the sensitization of these phenotypically resistant cells to the antiproliferative effects of SSAT upregulation by the bis(ethyl)polyamine analogues (Murray-Stewart et al. 2013). These data suggest the use of HDAC inhibitors in combination with SSAT-inducing polyamine analogues as an effective chemotherapeutic strategy in patients harboring clinically aggressive small-cell lung cancers.

Last, PMF1, which responds to increased polyamines and their analogues through NRF2-dependent transcriptional activation of *SAT1*, is frequently hypermethylated and silenced in human bladder tumors (Aleman et al. 2008). The degree of PMF1 methylation and the corresponding loss of PMF1 expression is significantly correlated with increased tumor stage and grade and is prognostic of poor overall survival. Furthermore, PMF1 hypermethylation is detectable in urinary specimens and can accurately distinguish bladder cancer patients from controls; a subsequent study indicated PMF1 methylation status as a predictor for patient response to a common bladder tumor therapy (Alvarez-Mugica et al. 2013). These data indicate a role for PMF1-mediated induction of SSAT in blocking the progression of bladder cancer, perhaps by maintaining intracellular polyamine levels below those necessary for tumor proliferation. Unfortunately, the downstream effects of PMF1 silencing on polyamine catabolism have not been reported.

5.3.2 Regulation by Infectious/Inflammatory Agents

The upregulation of polyamine catabolism in response to microbial infection and inflammatory stimulus is becoming a common theme. Several bacterial pathogens have now been identified to induce SMOX expression in host cells, the ultimate outcome of which is oxidative DNA damage, apoptosis, and an increased potential for neoplastic transformation. These pathogens include *Helicobacter pylori* and the enterotoxigenic *Bacteroides fragilis*, both of which are described in greater detail in the sections that follow (Xu et al. 2004; Goodwin et al. 2011). Additionally, a constituent of gram-negative bacterial cell walls, endotoxin (LPS), induces the transcription of both SSAT and SMOX in the kidney (Zahedi et al. 2010), and expression of an HIV1-encoded protein induces SMOX in neuronal cells (Capone et al. 2013).

Polyamine catabolism contributes to inflammation through the production of ROS; however, it is also regulated as a result of inflammatory signals. Both SMOX and SSAT are activated by the inflammatory cytokine tumor necrosis factor (TNF)-α; SMOX can be similarly induced by interleukin-6 (Babbar and Casero 2006; Babbar et al. 2006b, 2007). Furthermore, oxidative stress itself, in the form of H_2O_2, has been shown to induce *SAT1* transcription and alter intracellular polyamine concentrations (Chopra and Wallace 1998; Smirnova et al. 2012). Additionally, the alcohol metabolite acetaldehyde was recently shown to induce spermine oxidation (Uemura et al. 2013), ischemia–reperfusion injury induces both SSAT and SMOX catabolic pathways (Zahedi et al. 2009; Zahedi and Soleimani 2011), and exposure to carbon tetrachloride induces SSAT in hepatocytes (Zahedi et al. 2012). Each of these stimuli and their disease implications are discussed in the following sections.

5.4 Physiological Consequences of Increased Polyamine Catabolism

The higher polyamines, spermine in particular, play important physiological roles in protection from oxidative stress. Enhanced polyamine catabolism reduces this protection while concomitantly generating ROS and toxic aldehyde by-products. As a result, increased polyamine catabolism, resulting from the stimuli just mentioned, has been implicated in several pathophysiological conditions, including neurological and liver disease, stroke, kidney failure, and cancer.

5.4.1 ROS Generation and Oxidative DNA Damage

5.4.1.1 Inflammation-Associated Hyperproliferative Conditions

Approximately 20 % of all human cancers can be causally linked to chronic inflammation, particularly through infection with human pathogens (Zur Hausen 2009). Representatives of these pathogens are also inducers of SMOX, and elevated levels of SMOX have been observed in several inflammation-associated human conditions that are risk factors for the development of epithelial cancers. These observations suggest a role for spermine oxidation in the initiation of tumorigenesis. In addition to generating oxidative stress capable of DNA damage in the epithelial cell, several systems have now also demonstrated inductions of the polyamine oxidases in infiltrating inflammatory cells as a potential means for immune response evasion.

An active area of investigation regarding infection and inflammation focuses on *Helicobacter pylori*, a gram-negative bacterium that infects the stomach mucosa and causes inflammation in the form of chronic gastritis and peptic ulcers (Hardbower et al. 2013). Although eliciting acute and chronic immune and inflammatory responses, *H. pylori* evades the antimicrobial mechanisms of the immune response and often persists for the life of the host (Gobert et al. 2001; Bussiere et al. 2005). Approximately 50 % of the world's population is infected with *H. pylori*, which is considered a class I carcinogen and is believed to be the causal agent of 95 % of gastric cancers (Malfertheiner et al. 2005).

Gastric epithelial cells respond to infection with *H. pylori* through an induction of SMOX mRNA and activity. The generation of H_2O_2 that results from SMOX induction by *H. pylori* has been causally linked to DNA damage and apoptosis in the gastric mucosae of humans and mice (Xu et al. 2004). Importantly, a subpopulation of gastric epithelial cells in which *H. pylori* infection has induced SMOX activity and high amounts of DNA damage remains resistant to apoptosis, therefore increasing the likelihood that these cells will undergo malignant transformation (Chaturvedi et al. 2011). SMOX-mediated H_2O_2 production in response to *H. pylori* is also induced in the host macrophages responding to the infection, resulting in

macrophage apoptosis and contributing to immune evasion and bacterial persistence (Chaturvedi et al. 2004, 2013). Recent studies with affected patient samples have further implicated the critical role played by SMOX in the etiology of *H. pylori*-induced gastric cancer (Chaturvedi et al. 2014b, 2014c).

Chronic inflammation and the associated oxidative damage is also a risk factor for the development of colorectal neoplasia. Induction of SMOX has been demonstrated in a mouse model of infection with the enterotoxigenic bacterium *B. fragilis* (ETBF), which results in ulcerative colitis, acute diarrheal disease, inflammatory bowel disease, and ultimately causes colon cancer. This SMOX induction has been implicated as the source of ROS-induced DNA damage in ETBF-infected colonic epithelium, and treatment with the SMOX inhibitor MDL72, 527 decreased ETBF-induced DNA damage, colonic inflammation, proliferation, and tumorigenesis (Goodwin et al. 2011). In patient samples of ulcerative colitis, SMOX protein expression in infiltrating mononuclear cells was found to correlate with the severity of inflammatory disease scoring, consistent with a role for SMOX in colitis pathogenesis (Hong et al. 2010).

Chronic inflammation in the form of prostatitis is believed to contribute to the development of prostate cancer. In prostate tissue samples from patients with prostate disease spanning the spectrum from inflammation to prostate adenocarcinoma, SMOX protein expression was increased in comparison with individuals without disease (Goodwin et al. 2008). When examining patient-matched samples, SMOX expression was increased in prostatic intraepithelial neoplasia (PIN) and prostate cancer samples relative to benign prostatic epithelium from the same individual, with the greatest increase observed in PIN lesions, which are recognized as precursors to prostate carcinoma. Consistent with the inflammation-associated conditions already described, these data suggest a role for SMOX in precursor lesion development and carcinogenic initiation events. Furthermore, patients who developed prostate disease demonstrated significantly higher SMOX expression even in the nondiseased areas of prostatic epithelium when compared to those without disease, suggesting increased SMOX expression is an important component early in the disease process.

Pneumocystis infection is also associated with upregulated polyamine catabolism. *Pneumocystis* opportunistically infects the lungs of immunocompromised patients and results in a decrease in alveolar macrophages. This apoptosis appears to be the result of H_2O_2 production by the macrophages through increased APAO activity subsequent to the induction of polyamine biosynthesis (Liao et al. 2009).

5.4.1.2 Ischemia–Reperfusion and Toxin-Induced Injury

Renal ischemia–reperfusion has been shown to induce polyamine catabolism through SSAT and SMOX in both the kidney and liver, resulting in oxidative stress and apoptosis that do not occur in SSAT-deficient animals (Zahedi et al. 2009; Zahedi and Soleimani 2011). Furthermore, endotoxin, or LPS, is a major cause of sepsis-related acute kidney injury, and injection of mice with LPS resulted in inductions of renal SSAT and SMOX. Pharmacological inhibition of polyamine oxidation or ablation of SSAT decreased renal cell damage, implicating the catalysis of

polyamines in the mediation of endotoxin-induced acute kidney injury (Zahedi et al. 2010). In similar studies, it was demonstrated that exposing mice to carbon tetrachloride resulted in a large induction of hepatocyte SSAT activity that was associated with liver damage, and this damage was prevented by pharmacological inhibition of polyamine oxidation or SSAT ablation (Zahedi et al. 2012).

5.4.1.3 HIV-Related Dementia

Chronic oxidative stress occurs in brain tissues of HIV-infected patients and is associated with the development of human immunodeficiency virus (HIV)-associated dementia. Recently, the HIV-1 *Tat* gene was shown to induce SMOX activity, resulting in ROS generation and a reduction in intracellular spermine content in neuroblastoma cells. These studies provided evidence that SMOX-derived H_2O_2 induces oxidative stress that plays a role in neuronal cell death and the etiology of dementia associated with HIV infection (Capone et al. 2013).

5.4.2 Acrolein Generation

In addition to generating ROS, polyamine oxidation results in toxic aldehydes that have been implicated in neurological conditions and diseases (Pegg 2013). The aldehyde product of SMOX activity, 3-amidopropanal, can be spontaneously metabolized to acrolein, a major toxicity factor that is being actively investigated as a factor in several pathologies.

In this regard, multiple studies have implicated a role for polyamine oxidation-associated acrolein production in neuronal damage. APAO and SMOX proteins and acrolein adducts can be measured in the plasma of patients and hold potential as biomarkers for the detection of stroke (Igarashi and Kashiwagi 2011a, b; Tomitori et al. 2005). Acrolein conjugates, when combined with amyloid-β ratios, also provide an accurate indication for Alzheimer's disease, even in patients with only mild cognitive impairment (Waragai et al. 2012).

Increases in free and protein-conjugated acrolein have also been detected in various renal pathologies, including renal failure, and the metabolism of alcohol in the liver has been shown to induce spermine oxidation resulting in increased detection of acrolein (Sakata et al. 2003a, b; Uemura et al. 2013). This increase in hepatocellular SMOX suggests a mechanism for the liver toxicity and decreased regenerative abilities associated with chronic alcohol intake.

5.4.3 Reduction of Intracellular Polyamine Pools

The studies described here focus on the cellular effects of toxic by-products generated through elevated polyamine catabolism; however, the resulting reduction in intracellular polyamine concentrations must also be considered, as their depletion

can significantly exacerbate the toxic outcome. Spermine and spermidine function to protect DNA from oxidative damage, and spermine has been shown to act directly as a free-radical scavenger (Ha et al. 1998a, b; Khan et al. 1992a, b; Rider et al. 2007; Nilsson et al. 2000). Therefore, elevated polyamine catabolism, particularly through nuclear SMOX induction, not only generates ROS but also functionally reduces the antioxidant levels of the cell.

Similarly, polyamines have been recognized as mediators of immune function by negatively regulating the production of certain inflammatory cytokines, including TNF-α and interleukin (IL)-1β, and nitric oxide (Zhang et al. 2000; Perez-Cano et al. 2003; Paul and Kang 2013) Therefore, the transcriptional activation of SSAT and SMOX by pro-inflammatory cytokines, such as TNF-α, decreases the abundance of spermine and spermidine that would normally repress further production of TNF-α, thus having the potential to exacerbate the chronic inflammatory response.

5.5 Polyamine Catabolism as a Therapeutic Target

5.5.1 Chemotherapeutic Strategies

In the cancer setting, much research has focused on inducing polyamine catabolism in established tumors with the goal of reducing the natural polyamines required for tumor proliferation while selectively inducing tumor cell apoptosis through the generation of ROS (Nowotarski et al. 2013; Battaglia et al. 2013). Several classes of polyamine analogues have now been characterized to accomplish this goal in vitro; however, their clinical utility as single agents has been limited. Combining these analogues with other agents targeting the tumor cell has become a promising chemotherapeutic option. For example, cotreatment of small cell lung tumor cells with a bis(ethyl) polyamine analogue and the HDAC inhibitor MS-275 produced a synergistic induction of SSAT activity that was associated with growth inhibition (Murray-Stewart et al. 2013). Furthermore, these analogues have been observed to work in concert with other common cytotoxic agents, including 5-fluorouracil and platinum-based compounds, to induce polyamine catabolism (Hector et al. 2004, 2008; Pledgie-Tracy et al. 2010).

5.5.2 Chemopreventive Strategies

SSAT induction is observed in response to treatment with NSAIDs, and this induction contributes to the antiproliferative activity of NSAIDs in regard to the development of colorectal carcinoma (Babbar et al. 2003, 2006a). Recently, a phase III clinical trial examined the chemopreventive potential of combining treatment with a specific NSAID (sulindac) with an inhibitor of polyamine biosynthesis (difluoromethylornithine) (Meyskens et al. 2008). Patients in this study who received the combination therapy demonstrated dramatic reductions in the occurrence of colon

polyps and adenomas, verifying the utility of targeting polyamine metabolism in conjunction with inflammation.

In light of the growing evidence supporting a role for polyamine oxidation in the etiologies of several human conditions and diseases, including cancer initiation, attention must also be given to attenuating this response as a means for chemoprevention. As SMOX activity has been demonstrated in the cell nucleus, its inhibition in the presence of cancer-predisposing, SMOX-inducing factors such as *H. pylori* infection appears to provide protection from the oxidative DNA damage that leads to carcinogenesis. It is likely that this inhibition of polyamine catabolism prevents the generation of ROS while maintaining the intracellular spermine pools capable of free-radical scavenging. Although effective inhibitors for SMOX exist, including MDL72527, they are nonspecific and also inhibit APAO. The identification of specific inhibitors of the polyamine oxidases will provide both experimental and therapeutic benefit.

5.6 Summary

In conclusion, although consisting of only three enzymes, intracellular mammalian polyamine catabolism is a dynamic process that is capable of responding to a multitude of environmental stimuli. Depending on the context, these interactions can have positive or negative cellular effects: polyamine catabolism can protect cells from the toxic effects of excessive polyamine accumulation and limit uncontrolled proliferation in instances of upregulated polyamine biosynthesis. Yet, excessive polyamine catabolism can cause oxidative stress and toxin generation with the potential to increase carcinogenic events, limit the regeneration or viability of essential cells, or diminish the innate immune response to infection. Thus, both targeted increases in polyamine catabolism and targeted inhibition of polyamine catabolism have the potential for therapeutic benefit, depending on the precise context of the changes occurring. Additional studies are necessary to fully understand and exploit these dynamic pathways for maximum therapeutic advantage.

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