

CHAPTER 6

Ceramide in Stress Response

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Abstract

Evidence has consistently indicated that activation of sphingomyelinases and/or ceramide synthases and the resulting accumulation of ceramide mediate cellular responses to stressors such as lipopolysaccharide, interleukin 1β , tumor necrosis factor α , serum deprivation, irradiation and various antitumor treatments. Recent studies had identified the genes encoding most of the enzymes responsible for the generation of ceramide and ongoing research is aimed at characterizing their individual functions in cellular response to stress. This chapter discusses the seminal and more recent discoveries in regards to the pathways responsible for the accumulation of ceramide during stress and the mechanisms by which ceramide affects cell functions. The former group includes the roles of neutral sphingomyelinase 2, serine palmitoyltransferase, ceramide synthases, as well as the secretory and endosomal/lysosomal forms of acid sphingomyelinase. The latter summarizes the mechanisms by which ceramide activate its direct targets, PKC ζ , PP2A and cathepsin D. The ability of ceramide to affect membrane organization is discussed in the light of its relevance to cell signaling. Emerging evidence to support the previously assumed notion that ceramide acts in a strictly structure-specific manner are also included. These findings are described in the context of several physiological and pathophysiological conditions, namely septic shock, obesity-induced insulin resistance, aging and apoptosis of tumor cells in response to radiation and chemotherapy.

Introduction

Cells and organisms have developed various strategies to deal with adverse changes in their environment. Cellular insult by infectious agents, toxins, nutrient deprivation, or genotoxic stress produces a coordinated systemic response (generally referred to as inflammation), which is aimed at neutralization of the insult and initiation of tissue repair. The first line of defense at systemic level is stimulation of the innate immune response, which consists of regulated production of inflammatory mediators, including cytokines like IL- 1β , TNF α and IL-6. The typical cellular response to environmental stressors includes the induction of cellular apoptosis (in response to either genotoxic stress or cytokines like TNF α), growth arrest (during nutrient deprivation), increased eicosanoid production, cell migration and adhesion (in the presence of infectious agent). While acute inflammation is protective for the organisms, excessive and long-standing inflammation is harmful and underlies diseases like septic shock, atherosclerosis, asthma, rheumatoid arthritis and inflammatory bowel disease.

Diverse signaling pathways mediate cellular response to stress. The sphingolipid second messengers ceramide, ceramide-1-phosphate, sphingosine and sphingosine-1-phosphate play important role as mediators in many of these pathways. This chapter is focused on the role of ceramide in cellular stress response and the mechanisms of its generation and action.

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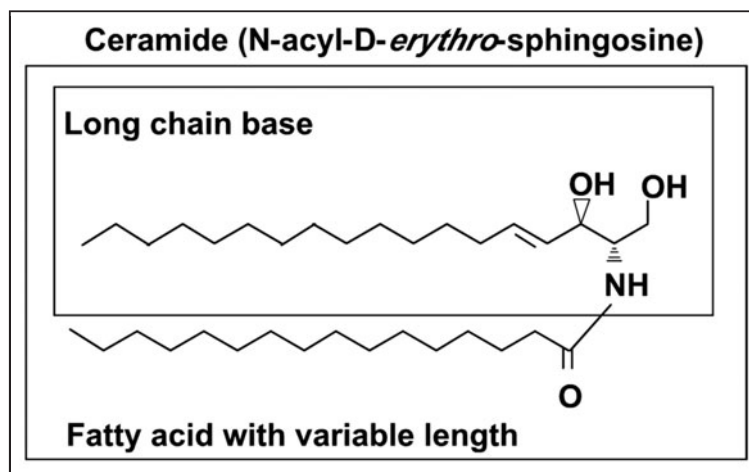


Figure 1. Structure of ceramide.

Chemical Structure and Biophysical Properties of Ceramide

Ceramides (Fig. 1) form the hydrophobic backbone of all complex sphingolipids¹ and consist of a long chain sphingoid base and amid linked fatty acid which is either saturated or unsaturated and vary in length from two to 28 carbon atoms. In mammalian cells, the most commonly found ceramides have *D-erythro*-sphingosine and a saturated fatty acyl chain of 16 carbon atoms and are among the most hydrophobic lipids in the membrane. Free ceramide has a very low critical micellar concentration ($\text{cmc} < 10^{-10}\text{M}$) and cannot exist in aqueous solutions.² Nevertheless, ceramides are still considered as amphiphiles because the hydroxyl group at the first carbon and the amide bond are hydrophilic moieties. Dihydroceramide differs from ceramide inasmuch as the latter contains a trans 4, 5 double bond, which is essential for some of the bioactive roles of ceramide.

The structural features in the ceramide molecule that are required for its biological properties are not well understood, however the two hydroxyl groups, amid group protons and the trans-double bond seems to be involved.³ A network of intramolecular hydrogen bonds involving the OH and NH groups establishes a unique conformation arrangement of ceramide molecule.

Changes in Ceramide Mass during Stress

The basal ceramide concentrations in the cells are low and may change during cellular differentiation or progression through the cell cycle. Various inducers of cellular stress however lead to an accumulation of ceramide that promotes apoptotic, inflammatory and growth inhibitory signals and also mediates the onset of a specific response. The data in Table 1 illustrate what is the magnitude of changes in ceramide levels under various conditions of stress. These reported differences however, are likely to be an underestimation since they are measured in total cell preparations and not in the specific membrane fractions where ceramide was generated. Furthermore, with the exception of mass spectrometry—based assays, the most commonly used methods of ceramide quantification do not separate the individual ceramide species.⁴ This might be important as the impact ceramide has on cell functions seemingly depends on the type of fatty acid attached to the sphingoid base. Recent data indeed support the notion that the different biological effects caused by ceramide may be mediated by distinct molecular species of the lipid, underscoring the necessity to evaluate changes in ceramide content in a structure-specific manner.

A comparison of the magnitudes of ceramide accumulation observed under various treatments reveals that the changes are more robust in response to chemotherapeutics or irradiation. As the outcome of these treatments has been the induction of cell death via apoptosis, some investigators

Table 1. Changes in ceramide content in response to stress and during some pathophysiological conditions

	Control	Treatment	Assay	Tissue/Cell Type	Ref.
Hypoxia/ Re- oxygenation	4.5 pmol/nmol LP	8.5 pmol/nmol LP	Mass Spec	NT-2	6
	100%	200% of control	DAGK	Cardiac myocytes	7
	22 pmol/nmol LP ^z	55 pmol/nmol LP	DAGK	NRK-52E	8
	250 pmol/mg Pr	900 pmol/mg Pr	DAGK	PC-12	9
	100%	300% of control	DAGK	HUVEC	10
	100%	50% of control	DAGK	A7r5	11
Ischemia/ Reperfusion	110% of sham	175% of sham	Mass Spec	Brain	12
	100%	200% of control	DAGK	Rat cardiomyocytes	13
	50 pmol/nmol	90 pmol/nmol LP	DAGK	Renal cortex	14
	190 pmol/mg Pr	290 pmol/mg Pr	DAGK	Liver	15
IL-1 β	100%	390% of control	DAGK	Rat astrocytes	16
	100%	170% of control	DAGK	CG-4	17
	18 pmol/nmol LP	45 pmol/nmol LP	DAGK	Rat myocytes	18
	75 cpm/10 ⁶ cells	160 cpm/10 ⁶ cells	Labeling	Rat mesangial cells	19
	1 nmol/mg Pr	1.5 nmol/mg Pr	HPLC	Rat hepatocytes	20
	1.1 nmol/mg Pr	1.48 nmol/mg Pr	HPLC	Rat hepatocytes	21
	250 pmol/10 ⁶ cells	500 pmol/10 ⁶ cells	DAGK	HUVEC	22
	450 pmol/10 ⁶ cells	800 pmol/10 ⁶ cells	DAGK	Dendritic cells	23
	100%	170% of control	Labeling	Thyroid cells	24
TNF- α	100%	150% of control	HPLC	Cortical neurons	25
	100%	330% of control	DAGK	Rat astrocytes	16
	200 pmol/mg Pr	850 pmol/mg Pr	DAGK	U-87 MG cells	26
	22 pmol/10 ⁶ cells	37 pmol/10 ⁶ cell	DAGK	Mesangial cells	27
	100%	300% of control	HPLC	Hepatocytes	28
	0.8 nmol/mg Pr	1.4 nmol/mg Pr	DAGK	Lung microsomes	29
	5.0 nmol/mg Pr	10.9 nmol/mg Pr	HPLC	Alveolar lavage	30
	0.5 nmol/nmol LP	1.1 nmol/nmol LP	Mass Spec	Endothelial cells	31
	100%	460% of control	DAGK	MIN6 cells	32
	100%	700% of control	DAGK	MCF-7 cells	33
	100%	175% of control	DAGK	MCF-7 cells	34
	100%	250% of control	DAGK	L929	35
	5 pmol/nmol LP	13 pmol/nmol LP	DAGK	U937 cells	36
5 pmol/nmol LP	12 pmol/nmol LP	DAGK	SK2	36	
Serum starvation	100%	200% of control	DAGK	Mouse keratinocytes	37
LPS	2.8 pmol/nmol LP	4.3 pmol/nmol LP	HPLC	Brain	38
	4 nmol/ml	8 nmol/ml	HPLC	Plasma	38
	3.2 nmol/ml	4.8 nmol/ml	HPLC	Mouse serum,	39
	100%	200-500% of contr.	HPLC	Human plasma	39
	3.5 nM/mg Pr	16 nM/mg Pr	HPLC	THP-1 cells	40
	258 pmol/mg Pr	1634 pmol/mg Pr	HPLC	Mouse M ϕ	41
	8 nM/mg Pr	22 nM/mg Pr	HPLC	Human alveolar M ϕ	42
	1.1 nmol/mg Pr	2.1 nmol/mg Pr	DAGK	Intestinal mucosa	43

continued on next page

Table 1. Continued

	Control	Treatment	Assay	Tissue/Cell Type	Ref.
ER stress	5 pmol/mg Pr	9 pmol/mg Pr	Mass Spec	INS-1 cells	44
Ionizing radiation	100%	700% of control	DAGK	OLG	45
	100%	320% of control	Labeling	CHK cells	46
	0.95 nmol/10 ⁶ cells	1.25 nmol/10 ⁶ cells	DAGK	BAEC	47
	6-12 nmol/ml	9-18 nmol/ml	HPLC	Human serum	48
	100%	250% of control	Labeling	Keratinocytes	49
	10 pmol/10 ⁶ cells	50 pmol/10 ⁶ cells	Mass Spec	Jurkat cells	50
	100%	130% of control	DAGK	HEK293	51
	100%	140% of control	DAGK	Jurkat T-cells	51
	10 pmol/nmol LP	17 pmol/nmol LP	Mass Spec	MCF-7	52
	100%	320% of control	DAGK	HeLa	53
	100%	140% of control	Labeling	U937 cells	54
	100%	900% of control	DAGK	Human platelets	54
	Diabetes/obesity	0.5 pmol/μg Pr	1.0 pmol/μg Pr	DAGK	Rat heart tissue
12 pmol/mg tissue		23 pmol/mg tissue	Mass Spec	Muscle	56
25 pmol/mg tissue		40 pmol/mg tissue	DAGK	Muscle	57
0.2 nmol/mg tissue		0.3 nmol/mg tissue	DAGK	Liver	57
15 pmol/mg		23 pmol/mg	Mass Spec	Muscle	58
100%		No change	Mass Spec	Liver	58
3.5 pmol/μl		9 pmol/μl	Mass Spec	Serum	58
0.5 nmol/mg Pr		1.5 nmol/mg Pr	HPLC	Liver	59
2.4 nmol/ml		3.1 nmol/ml	Mass Spec	Human plasma	60
120 pmol/150μl		190 pmol/150μl	Mass Spec	Mouse Serum	61
380 pmol/mg Pr		580 pmol/mg Pr	Mass Spec	Epididymal fat	61
300 pmol/mg Pr		550 pmol/mg Pr	Mass Spec	Subcutaneous fat	61
650 pmol/ml		1400 pmol/ml	Mass Spec	Mouse plasma	62
310 pmol/mg Pr		210 pmol/mg Pr	Mass Spec	Epididymal fat	62
5 nmol/g		8 nmol/g	Mass Spec	Subcutaneous fat	63
0.6 nmol/mg Pr	1.2 nmol/mg Pr	DAGK	Pancreatic islets	64	
250 pmol/mg Pr	180 pmol/mg Pr	Mass Spec	Retina	65	
Aging	100%	170% of control	Mass Spec	Cerebral cortex	66
	100%	220% of control	DAGK	Heart	67
	175 pmol/μg DNA	250 pmol/μg DNA	Mass Spec	Adipose tissue	68
	0.45% of total LP	0.8% of total LP	HPLC	Liver	69
	1.3 nmol/mg Pr	1.7 nmol/mg Pr	HPLC	Rat hepatocytes	70
	0.5 pmol/mg Pr	1.1 pmol/mg Pr	DAGK	Endothelium	71
	3.5 pmol/nmol LP	6.0 pmol/nmol LP	DAGK	WI-38 HDF	72
Alzheimer's disease	27 nM/ml	52 nM/ml	DAGK	CSF	73
	100%	175% of control	Mass Spec	Brain	74
	2.5 nmol/mg Pr	9 nmol/mg Pr	Mass Spec	Temporal cortex	75
	2.0 nmol/mg Pr	8 nmol/mg Pr	Mass Spec	Cerebellum	75
Heat stress	1.5 pmol/nmol LP	3.8 pmol/nmol LP	DAGK	Molt-4	76

continued on next page

Table 1. Continued

	Control	Treatment	Assay	Tissue/Cell Type	Ref.
Oxidative stress	100%	800% of control	DAGK	Human primary	77
	100%	200% of control	DAGK	OLG	78
	100%	170% of control	DAGK	Cerebral cortex	79
	100%	200% of control	DAGK	PC12	80
	100%	350% of control	Mass Spec	Airway epithelium	81
	100%	140% of control	Labeling	APRE-19	82
	100%	180% of control	DAGK	SMC HAEC	83
Car-diovascular disease	210.5 pmol/mg Pr	305.9 pmol/mg Pr	DAGK	Mouse heart	84
	100%	140% of control	Mass Spec	Mouse heart	85
	135 nmol/ml	220 nmol/ml	Mass Spec	Plasma	86
Anti-cancer therapies	100%	1000% of control	Labeling	LnCaP	87
	100%	200% of control	Labeling	PC-3	87
	7 pmol/nmol LP	70 pmol/nmol LP	DAGK	MDA-MB 231	88
	100%	220% of control	Labeling	MDA-MB 468	89
	100%	350% of control	Labeling	MCF-7	89
	1% of total LP	10% of total LP	Labeling	MCF-7	90
	100%	900% of control	Labeling	MCF-7	91
	100%	180% of control	Labeling	BT-20	92
	100%	300% of control	Labeling	MDA-MB 231	92
	100%	650% of control	Labeling	MDA-MB 468	92
	100%	300% of control	Labeling	Hs-578T	92
	100%	380% of control	Labeling	T47D	92
	100%	620% of control	Labeling	MCF-7	92
	100%	700% of control	Labeling	HL-60/VCR	93
	100%	220% of control	Labeling	U-937	93
100%	220% of control	Labeling	CHLA-90	94	
0.6 ng/10 ⁶ cells	1.8 ng/10 ⁶ cells	DAGK	JHU-022	95	

In some cases numerical values were taken from graphically represented data and were rounded to the nearest whole number. Abbreviations: General: CSF, cerebrospinal fluid; DAGK, diacylglycerol Kinase; LP, lipid phosphate; Mø, primary macrophages; OLG, oligodendrocytes; Pr, Protein. Cells lines: A7r5, rat embryonic thoracic aorta smooth muscle; APRE-19, human retinal pigment epithelium; BAEC, bovine aortic endothelium; BT-20—human breast carcinoma; CG4, oligodendrocyte progenitor; CHK, Chinese hamster kidney; CHLA-90, human neuroblastoma; HAEC, human airway epithelial; HDF, human diploid fibroblasts; HEK 293, human embryonic kidney; HeLa, cervical carcinoma; HL60/VCR, drug resistant human leukemia; Hs-578T, breast carcinoma; HUVEC, human umbilical vein endothelium; INS-1, rat insulinoma; JHU-022, squamous cell carcinoma; Jurkat, T-lymphocytes; L929, mouse fibroblasts; MDA-MB 231, human Caucasian breast adenocarcinoma; MDA-MB 468, human Black breast adenocarcinoma; MCF-7, human breast adenocarcinoma; MIN6, murine beta cells; Molt-4; human acute lymphoblastic leukemia; LnCaP, human prostate carcinoma; NRR-52E, rat kidney epithelium; NT-2, human neuronal precursor; PC3, human prostate carcinoma, PC12, rat adrenal medulla carcinoma; SK2, hybridoma; SP-1, mammary intraductal adenocarcinoma; T47D, human ductal breast epithelial tumor; TNP-1, human acute monocytic leukemia; U87, human astrocytoma; U937, human leukemic monocyte lymphoma.

suggest that ceramide plays the role of a gauge that senses the level of cell injury and depending on downstream factors determines specific biological outcome.⁵

Mechanisms for Ceramide Generation during Stress

Ceramide does not move spontaneously between cellular membranes and is transported either during normal membrane biogenesis, or with the aid of CERT, a protein that transfers ceramides synthesized at the endoplasmic reticulum to the Golgi apparatus. This insolubility of ceramide suggests that once generated, ceramide is likely to remain localized at the place of its synthesis until metabolized to other sphingolipids. Consequently, the increases in ceramide concentration during stress response are compartmentalized in distinct locations within the cells and might affect diverse sets of downstream targets and responses.

The two main metabolic pathways for generation of excess ceramide during stress response are (i) the de novo synthesis in the endoplasmic reticulum and (ii) the turnover of sphingomyelin (SM) either at the plasma membrane or in the endosomal/lysosomal compartment (Fig. 2). A number of agonists have been shown to activate these pathways leading to transient elevation in ceramide. The magnitude and temporal pattern of ceramide accumulation is further influenced by the activity of ceramidases, SM synthases, ceramide kinase and glucosyl/galactosyl ceramide synthases. These enzymes catalyze the conversion of ceramide to other sphingolipids and some agonists seem to coordinately regulate both, the ceramide production and turnover.^{20,96}

Role of the De Novo Pathway for Ceramide Generation in Cellular Stress Response

The de novo pathway for synthesis of ceramide consists of 4 reactions: the serine palmitoyl-transferase (SPT), which condenses palmitoyl-CoA and serine into 3-ketosphinganine, the 3-ketosphinganine reductase that generates sphinganine, the (dihydro)ceramide synthase, which

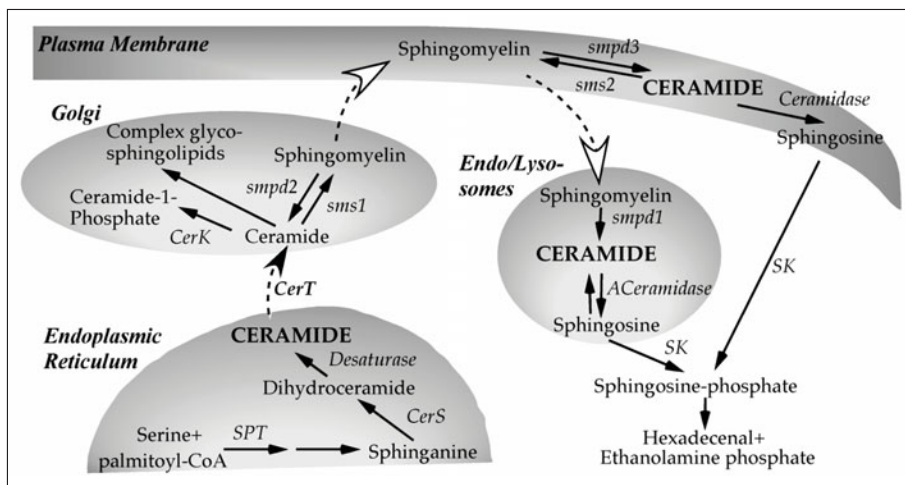


Figure 2. Metabolic pathways responsible for ceramide synthesis and degradation. The pools of ceramide involved in cellular response to stress are depicted in capital letters. Name of the enzymes are in *plain italic*. The names of relevant subcellular organelles are shown in **bold italic**. Black solid arrows are used to depict metabolic conversions. Black dashed arrow indicates protein-mediated transfer. White dashed arrow indicates vesicular transport.

Abbreviations: SPT: Serine Palmitoyltransferase; CerS: Ceramide synthases; CerT: ceramide transfer protein. SMS1 & 2: sphingomyelin synthase 1 & 2; smpd1: Acid Sphingomyelinase, smpd2: Neutral sphingomyelinase1, smpd3: Neutral sphingomyelinase 2. CerK1, Ceramide Kinase1; SK: sphingosine Kinase. ACeramidase: Acid Ceramidase.

acylates sphinganine to dihydroceramide and the dihydroceramide desaturase, which converts relatively inactive dihydroceramides to ceramides. Stimulation of the *de novo* pathway during cellular stress response happens through up-regulation of the activity of SPT and/or (dihydro) ceramide synthase. The enzymes of the *de novo* synthesis of ceramide are in the endoplasmic reticulum. The newly generated ceramide is actively transported to the Golgi apparatus, where it serves as a rate-limiting substrate in the synthesis of complex sphingolipids, like SM and glycosphingolipids. It is noteworthy that some of the known ceramidases may also act as ceramide synthases in a CoA-dependent and independent manner as they exhibit reverse activity. This alternative route for ceramide synthesis is often referred to as the salvage pathway and its role in stress response is not well understood.

The activation of the *de novo* pathway apparently leads not only to an elevation in ceramide but also to increases in the concentration of SM and glycosphingolipids. Such parallel accumulation is documented in response to LPS, palmitate treatment and heat shock, among others and implies that a wide range of cell functions might be affected through specific and nonspecific mechanisms. Inhibitors of glucosylceramide synthase and labeling with radioactive precursors have been successfully used to elucidate the specific role of ceramide in each case.

De novo synthesis of ceramide is target of a number of fungal inhibitors like fumonisin B1, which inhibits dihydroceramide synthase and the reverse reaction of some ceramidases⁹⁷ and myriocin/ISP-1,⁹⁸ cycloserine⁹⁹ and beta-chloroalanine,¹⁰⁰ all of which inhibit SPT. These widely used inhibitors remain a critical test of establishing whether the *de novo* pathway is involved in a particular cellular response (reviewed in Merrill¹⁰¹ and in Perry⁹⁶).

The activation of the *de novo* pathway during conditions of stress was initially discovered in yeast, where it regulates growth and the response to heat or osmotic stress (reviewed in Dickson¹⁰² and in Meier et al¹⁰³). However, in yeast the signaling mediator is not ceramide but the free long chain bases, phytosphingosine and dihydrosphingosine, most likely reflecting the specifics of sphingolipid synthesis in yeast. Later, the activation of the *de novo* pathway in *C. Elegans* in response to ionizing radiation was described and found to be involved in activation of the CED-3 caspase¹⁰⁴ and the resulting apoptosis of germ cells.

In mammalian systems, the *de novo* pathway seems to play a prominent role in cellular response to heat or chemical stress,¹⁰⁵ septic shock,¹⁰⁶ lipo-apoptosis¹⁰⁷ and insulin resistance,¹⁰⁸ as well as in receptor-dependent and -independent induction of apoptosis by variety of chemotherapeutic agents like etoposides¹⁰⁹ and doxorubicin.¹¹⁰

Heat Stress

In the human acute lymphoblastic leukemia cell line Molt-4, heat shock induces more than twofold increase in total ceramide levels with C16 ceramide being the major species affected. This accumulation of ceramide has been linked to the induction of c-jun and apoptosis.¹⁰⁵ Labeling with tritiated palmitate has shown an accumulation of ceramides, but not sphingoid bases, thus confirming that in contrast to yeast, where sphingoid bases mediate heat shock, in mammals, ceramide is apparently involved. Both, myriocin and fumonisin B1 inhibit the increase in total ceramide mass thus confirming that ceramides produced upon heat shock were products of SPT and ceramide synthase activity.

Septic Shock

Similar observations delineate a role of the *de novo* synthesized ceramide in septic shock *in vitro* as well as *in vivo*.¹⁰⁶ Administration of LPS or cytokines to rabbits increases hepatic sphingolipid biosynthesis leading to the accumulation of ceramide, SM and glycosphingolipids. Studies in cell lines link the stimulation of the *de novo* pathway to the activation of the MAP kinases and NFκB and respectively to the innate immune response. In nonhepatic tissues the elevation in ceramide biosynthesis during septic shock is correlated with the elevated glycosphingolipid synthesis that seems to play a role in pathogen recognition.

Activation of ceramide synthesis in the liver has been linked to production and secretion of lipoproteins enriched in ceramides, sphingomyelins and glycosphingolipids. The functions of

these lipoproteins with “altered” sphingolipid content was not well understood; it became clear however that the activation of the *de novo* synthesis during septic shock is paralleled by activation of another ceramide-generating enzyme, the Zn²⁺-dependent secretory form of ASMase, termed SSMase. This enzyme hydrolyses SM in the Low Density Lipoproteins (LDL), leading to a robust increase in LDL ceramide content. Several lines of evidence suggest that LDL that are rich in ceramide might mediate injury to the arterial wall during inflammation. LDL extracted from atherosclerotic plaques contain higher ceramide content as compared to LDL isolated from the plasma.¹¹¹ Experimentally-induced elevation in LDL ceramide content has been further linked to higher rate of LDL aggregation¹¹² and oxidation, as well as with an increased potential to induce apoptosis in microvascular endothelial cell.¹¹³

Lipotoxicity and Insulin Desensitization

Consumption of diet rich in saturated fats (also known as Western diet) is the main risk factor for the development of insulin resistance, hyperglycemia and atherosclerosis. Palmitic acid is the main component of the Western diet and is linked to excessive accumulation of lipids in lean tissues, mainly muscle and liver, lipotoxicity and insulin resistance. Because SPT has almost exclusive preference for the CoA-thiol ester of palmitate, numerous studies have investigated whether Western diet affects *de novo* synthesis of ceramide. These studies have shown that excess palmitate (delivered via the consumption of Western diet, *i.v.* infusion, or directly added to cells in culture) stimulates the flux through the *de novo* pathway resulting in accumulation of ceramide, SM and glycosphingolipids.^{58,114} Inhibition of SPT by myriocin prevents not only the palmitate flux through the pathway but also inhibits lipotoxicity, improves insulin response and leads to better glucose regulation.

The exact mechanism by which ceramide affects insulin response is not completely understood. Schmitz-Peiffer et al¹¹⁵ observed that the accumulation of ceramide in myotubes exposed to palmitate was paralleled by inhibition of Akt/PKB. Cotreatment with myriocin, cycloserine, or fumonisin B1 restored insulin-stimulated Akt phosphorylation, even in the presence of excess palmitate, suggesting that palmitate-induced stimulation of *de novo* synthesis is required for inhibition of insulin responsiveness. Recent data however imply that *in vivo*, the diet-induced ceramide increases have to be accompanied by increased triacylglycerides synthesis and accumulation in order to affect the overall insulin response. Furthermore, stimulation of the *de novo* synthesis of sphingolipids and that of triacylglycerides seems to be correlated in liver.⁵⁹

Programmed Cell Death

Perhaps, the most extensively studied cellular response to ceramide is the induction of programmed cell death or apoptosis. Activation of (dihydro)ceramide synthase in particular is implicated in endothelial cell death induced by TNF α ,¹¹⁶ in daunorubicin,¹¹⁰ doxorubicin and gemcitabine-induced apoptosis and may account for some aspects of the toxicity of phorbol esters,¹¹⁷ angiotensin II,¹¹⁸ cannabinoids¹¹⁹ and ischemia-reperfusion. In the latter case, investigation of intracellular sites of ceramide accumulation reveals that the elevation of ceramide is in mitochondria and is caused by the activation of a mitochondrial ceramide synthase via posttranslational mechanisms. Furthermore, ceramide accumulation appears to cause mitochondrial respiratory chain damage that could be mimicked by exogenously added natural ceramide to mitochondria.¹²

The recent cloning and characterization of 6 members of the ceramide synthase family CerS1-6 (also known as longevity assurance gene 1-6 (LASS1-6)) provided the opportunity to finally begin studying the role of these enzymes in apoptosis in more details. CerS1 but not CerS2-6 for example, was found to be sufficient and required for apoptosis in response to gemcitabine/doxorubicin treatment.¹²⁰ Notably, the gemcitabine/doxorubicin combination treatment was discovered to increase only the levels of C18-ceramide, the preferable substrate for CerS1. These and other studies have provided experimental evidence to the earlier assumptions that not only the type of sphingoid base (*i.e.*, sphingosine vs sphinganine) but also the fatty acid length and degree of saturation influences the biological effectiveness of ceramide.

The role of the de novo pathway in apoptosis had also been investigated in different animal models. Inhibition of the enzymes controlling the de novo pathway prevents alveolar cell apoptosis, oxidative stress and emphysema (the prevalent disease caused by cigarette smoking) in both rats and mice,¹²¹ delays the progression of atherosclerotic plaques in mice¹²² and ameliorates some of the pathological consequences of spinal cord injury.¹²³

Autophagy

The final paradigm to be discussed is the role of the de novo synthesis of ceramide in autophagy, an evolutionary conserved cytoprotective mechanism that sustains cells during periods of nutrient limitation, but under certain conditions may lead to mammalian cell death. Ceramide addition is sufficient to induce autophagy in some cells and a correlation between the rate of autophagy and ceramide synthesis has been observed in same pathological conditions suggesting a mechanistic link between ceramide, autophagy and disease (reviewed in Zheng et al²). Activation of de novo ceramide synthesis mediates autophagy in response to a bioenergetic crisis resulting in the rapid and profound down regulation of nutrient transporter proteins.¹²⁴ Ceramide is also shown to mediate the tamoxifen-dependent accumulation of autophagic vacuoles in the human breast cancer MCF-7 cells and to counteract interleukin 13-dependent inhibition of macroautophagy in HT29 cells.¹²⁵ The mechanism seems to involve the pro-autophagic protein beclin 1.

Mechanisms of Activation of De Novo Synthesis of Ceramide during Stress

Regulation of SPT at a transcriptional level has been seen with a number of agents, including endotoxin and cytokines,¹⁰⁶ UVB irradiation¹²⁶ and others.¹²⁷ Induction of both form of SPT, SPT1 and SPT2, occurs in balloon-injured rat carotid artery.¹²⁸ In contrast, long-term consumption of food rich in palmitate is correlated with increases in SPT1 protein but not mRNA level.⁵⁹ Activation of SPT occurs also posttranslationally in response to etoposide³³ and to heat shock in yeast.³⁴ Mitochondrial injury in cerebral ischemia/reperfusion activates ceramide synthases via posttranslational mechanism which is dependent of JNK.¹² Cers1 mRNA transcription is up-regulated in response to a gemcitabine/doxorubicin combination treatment,¹²⁰ while cisplatin is shown to cause a specific translocation of Cers1 from the endoplasmic reticulum to the Golgi apparatus.¹²⁹ The de novo pathway is also modulated through a negative feedback mechanism determined by the rate of sphingolipid degradation in the lysosomes since lipoproteins, sphingosine phosphate¹³⁰ and ASMase activity⁵⁹ seems to inhibit the flux through it. This might be a mechanism to prevent excessive synthesis of ceramide during normal healthy state of the cell.

Role of the Sphingomyelinases in Cellular Stress Response

The SMase family is a group of biochemically and genetically different enzymes all of which hydrolyze SM to ceramide and phosphorylcholine. SMase activities with neutral and acidic pH optima are found in most mammalian cells and an enzyme active in alkaline pH is localized in the intestinal wall. Currently, research is focused on 4 genes encoding different mammalian SMases: *smpd1* encodes two forms of acidic SMase, one associated with the endosomal/lysosomal compartment (ASMase) and a second one found in the plasma and the conditioned medium of stimulated cells (SSMase). *smpd2* and *smpd3* encode the Neutral SMase 1 (nSMase1) and 2 (nSMase2), both of which are Mg²⁺-dependent but differ in their subcellular localization and role in signaling. Data from several labs have shown that in mammalian cells, nSMase1 is a housekeeping enzyme with no particular function in signaling. The recently cloned zebra fish nSMase1, however seems to mediate heat-induced apoptosis in zebra fish embryonic cells.¹³¹ In mammalian cells, nSMase2 is regulated by cytokines like IL-1 β and TNF α and mediates some of the cytokine effects.¹³²⁻¹³⁴ The recently cloned *smpd4*, is suggested to encode a novel form of NSMase, nSMase3 that is found predominantly in skeletal muscle and heart.

Neutral Sphingomyelinase

Hepatic Acute Phase Response

The acute phase response of liver is an essential component of the systemic host response to bacterial infection and injury and requires IL-1 β , a prototypic inflammatory cytokine. Activation of NSMase by IL-1 β resulting in transient elevation in ceramide concentration is observed in number of cells including hepatocytes, mesangial cells, EL-4 cells and it has been related to activation of TAK-1, JNK and NF- κ B, all of which have important roles in the IL-1 β cascade.¹³⁵⁻¹³⁷

In hepatocytes, specific silencing of nSMase2 with siRNA has only a minimal effect on the basal cellular NSMase activity, however it results in a complete inhibition of the IL-1 β -stimulated NSMase activity.¹³⁸ Therefore, nSMase2 is probably an inducible enzyme that contributes little to the basal turnover of SM, but at the same time it is also the only neutral SMase activated by IL-1 β . The role of NSMase2 in the IL-1 β signaling cascade is rather complex: seemingly, the activation of nSMase2 modulates the pattern of phosphorylation of JNK by IL-1 β and respectively the magnitude of transcriptional induction of the hepatic acute phase proteins like C-Reactive Protein, α 1 Acid Glycoprotein and Insulin-like Growth Factor Binding Protein-1. nSMase2 activation in hepatocytes leads to potentiation of JNK phosphorylation due to stabilization of the IL-1 β receptor-associated kinase-1. The latter most likely involves a phosphatase, like the ceramide-activated protein phosphatase 2A (PP2A).¹³⁹

Vascular Inflammation

Activation of nSMase2 by another pro-inflammatory cytokine, TNF α is well documented in cells of the vasculature and various cancer cells.^{132,140,141} TNF α -induced nSMase2 activation is a prerequisite for endothelial nitric oxide synthase activation in HUVEC cells,¹³⁴ as well as for the up-regulation in A549 lung epithelial cells of vascular cell adhesion molecule and intracellular adhesion molecule 1,¹⁴¹ all of which have prominent roles in vascular inflammatory responses.

Apoptosis

Studies into the activation of NSMase during apoptosis have focused mainly on the role of NSMase-mediated ceramide production in the apoptosis induced by 55 kDa receptor for TNF α .¹⁴² A protein factor associated with NSMase activation, FAN, which interacts with the membrane-proximal domain of the p55 receptor and couples stimulation of the receptor to neutral SMase activation, is required for TNF α -induced ceramide generation, caspase processing and apoptosis.¹⁴³ Subsequent work in MCF-7 cells found that activation of NSMase in TNF α -stimulated cell death is upstream of mitochondrial changes, cytochrome C release and caspase-9 activation.¹⁴⁴

Oxidative stress-induced cell death may also be attributed to activation of NSMase. Free oxygen (H₂O₂), but not nitrogen (ONOO⁻) radicals specifically activate nSMase2, while silencing nSMase2 prevents H₂O₂-induced apoptosis, but had no effect on ONOO⁻-induced apoptosis.⁸³

Very recent studies had began to suggest that a novel form of neutral SMase, nSMase3 is a DNA damage and nongenotoxic stress-regulated gene that is deregulated in human malignancies and modulate the sensitivity of cancer cells to adriamycin-induced cell killing.¹⁴⁵ Early studies seem to suggest that at least the overexpressed nSMase3 can be activated by TNF α within seconds of stimulation.

Growth Arrest

A number of studies have established a role for NSMase-generated ceramide in regulating the cell cycle and mediating growth arrest, possibly through dephosphorylation of retinoblastoma protein and/or regulation of cyclin dependent kinases. Serum withdrawal causes activation of NSMase and cell cycle arrest at G0/G1 in Molt-4 cells.¹⁴⁶ Interestingly, a study focusing on genes up-regulated during confluence-induced arrest had initially identified nSMase2 as a confluence-arrest gene, CCA1, in rat 3Y1 cells.¹⁴⁷ In line with that, nSMase2 seems to mediate confluence-induced growth arrest of MCF-7 cells. The latter was preceded by confluence-induced translocation of nSMase2 to the plasma membrane.¹⁴⁸

Aging and Cancer

Constitutive up-regulation of nSMase2 and elevation of ceramide concentration during aging has been observed in liver, brain, macrophages and other tissues.¹⁴⁹ This aging-associated elevation in ceramide seems important for the onset of aging process since it has been causatively linked to hyperresponsiveness to IL-1 β ⁷⁰ and LPS⁴¹ and to deregulation of nitric oxide production in endothelium.⁷¹ A substantial decline in hepatic GSH content, which is characteristic for the aging process is responsible for this constitutive increase in nSMase2 activity.¹⁵⁰ nSMase2 apparently follows a pattern of regulation consistent with “developmental-aging” continuum, since in animal models of delayed aging, like calorie-restricted animals, the aging-associated changes in NSMase activity and function are reversed.¹⁵⁰ In cellular model of senescence, similar induction of NSMase activity and accumulation of ceramide has been observed in senescent cells and linked to the decline in proliferative capacity and onset of senescence.⁷²

In contrast, a somatic homozygous deletion specifically targeting nSMase2 (Smpd3) is found in a genomic screen for gene copy losses contributing to tumorigenesis in a mouse osteosarcoma model, while loss-of-function mutations in smpd3 gene were identified in 5% of acute myeloid leukemia and 6% of acute lymphoid leukemia cancers. It has been suggested that the mutation could be linked to a defect in plasma membrane translocation of nSMase2 and decreased responsiveness to TNF α . Reconstitution of smpd3 expression in mouse tumor cells lacking the endogenous gene enhances the TNF α -induced reduction of cell viability.¹⁵¹

Mechanisms of Activation of NSMase

- (i) **Translocation:** Studies have shown that nSMase2 can be translocated to the plasma membrane when cell confluence is reached.¹³² This translocation of nSMase2 is also required for the confluence-induced cell cycle arrest to ensue.¹⁵² In oligodendroma-derived cells, a regulated translocation of NSMase2 to the caveolae, which are the signaling domains of the plasma membrane¹⁵³ has been observed, while studies with highly differentiated nonproliferating primary hepatocytes have shown that the overexpressed nSMase2 is localized constitutively at the plasma membrane.¹³³ These data suggest that translocation of nSMase2 to the plasma membrane might be important mechanisms for regulation of its activity *in situ* by bringing the enzyme into contact with its substrate. Pharmacological inhibitors and specific siRNA has implicated the novel PKC, specifically PKC δ , in TNF and PMA-stimulated nSMase2 translocation to the plasma membrane.⁵²
- (ii) **NSMase as a redox-sensitive enzyme.** The major scavenger of reactive oxygen species, GSH, has been found to be a reversible inhibitor of cellular NSMase activity.^{33,154} This is noteworthy, because depletion of cellular GSH content is observed in conditions of increased oxidative stress. The modulation of NSMase activity by GSH was first established in the context of regulation of TNF α signaling and apoptosis.^{33,154} Later, the ability of GSH to affect the sensitivity of T47D/H3 breast cancer cells to doxorubicin was attributed to the inhibitory effect GSH has on NSMase activity.¹⁵⁵ A correlation between oxidative stress and NSMase activity was also found in long-lived rats on vitamin Q10 enriched diet¹⁵⁶ and in astrocytes treated with vitamin E.¹⁵⁷ Finally, recent research has shown that specific downregulation of nSMase2 with siRNA blocks H₂O₂-induced apoptosis of human aortic endothelial cells, identifying nSMase2 as a redox-sensitive protein.¹⁵⁸ Detailed analyses of sensitivity of nSMase2 to GSH in hepatocytes suggest that GSH depletion exerts its effect on NSMase activity following a sigmoid dose dependent curve. A rapid and significant activation of NSMase is observed only when hepatic GSH concentration drops below 30% of its basal level, implying that there is a threshold required for NSMase activation during oxidative stress.¹⁵⁰ Interestingly, nSMase1 is also sensitive to changes in the GSH/GSSG ratio.¹⁵⁹ This suggests that redox sensitivity might be a common property of neutral SMases.

Acid Sphingomyelinase

Endotoxic Shock

Patients with severe sepsis exhibit an enhanced SMase activity in plasma. The increase is correlated with the severity of illness and the fatal outcome.¹⁶⁰ Studies in humans and mice administered with LPS confirm these observations.³⁹ Deletion of ASMase protects against LPS-induced elevation in the plasma SMase activity¹⁶¹ and attenuates endothelial apoptosis and animal death⁴³ implicating a secretory isoform of ASMase (SSMase) in apoptosis and organ failure in sepsis. The physiological and biochemical properties of this SSMase are not well understood. It has been postulated that SSMase degrades SM in the secreting cell outer membrane leaflet, which contains almost ¾ of the cellular SM. This SM pool is seemingly inaccessible for other cellular SMases, since the active center of nSMase2, the sole plasma membrane-associated SMase, is facing the cytosolic leaflet of the membrane. These proposed autocrine functions of SSMase however had not been rigorously tested. In turn, substantial evidence had implicated SSMase in modifying the SM/ceramide content of circulating LDL as discussed earlier in the chapter.

Apoptosis (reviewed in ref. 162)

Irradiation of tumor cells with ionizing radiation^{52,163-165} transiently activates ASMase with maximal activity detected between 1 to 10 min post irradiation. The activation of ASMase has been linked to the ability of radiation to induce apoptosis since ASMase deficiency causes apoptosis resistance in various tissues in ex vivo or in vivo experiments.¹⁶³⁻¹⁶⁵ Genetic restoration of the activity also restores the radiation effects indicating that ASMase mediates apoptosis via ceramide, at least in some cells including B cells, endothelial cells or mesothelium, lung epithelial cells, MCF-7 breast cancer cells, etc. However, some other cell types, for example, thymocytes, remained sensitive to radiation in ASMase-deficient mice suggesting that radiation effects are mediated by different mechanisms depending on the cell type.

ASMase is also involved in death receptor-mediated apoptosis. Stimulation via the CD95 receptor leads to ASMase activation and ceramide accumulation that precedes the induction of cell death.¹⁶⁶⁻¹⁶⁸ Furthermore, studies with fibroblasts from Niemann–Pick disease type A patients, who exhibit deficiency of ASMase and hepatocytes from ASMase null mice show that ASMase activation is required and sufficient for CD95 induced apoptosis.^{168,169}

Viral and Bacterial Infections

Cellular ASMase activity seems to play an important role in susceptibility of mammalian organisms to microbial infections. ASMase-deficient mice were found to be more sensitive to infections with the Gram-negative bacteria *L. monocytogenes*, due to a defect in lyso-phagosomal fusion. In wild-type macrophages, the phagosome rapidly fuse with the lysosomes to form a phago-lysosome and to kill and digest bacteria, while in macrophages deficient in ASMase the process is slower and leads to inefficient transfer of lysosomal antibacterial hydrolyses into phagosomes.¹⁷⁰ Apparently, ASMase is required for the proper fusion of late phagosomes with lysosomes.¹⁷¹ ASMase-deficient mice were also more susceptible to infection with Sindbis virus, an enveloped virus with a single-stranded RNA that is involved in fatal alpha virus encephalomyelitis, due to more rapid replication and spread of the virus in the nervous system.¹⁷²

Mechanisms of Activation of ASMase

One mechanism for activation of ASMase involves its phosphorylation by PKC δ at Ser⁵⁰⁸ which mediates UV light-induced ASMase activation and cell death in MCF-7 breast cancer cells.⁵² Phosphorylation of ASMase seems to be correlated with its translocation to the plasma membrane (see below) in irradiated cells.

Evidence for a Coordinated Regulation of Multiple Pathways for Ceramide Generation

A handful of studies suggest that the activity of the two SMases and the de novo pathway might be regulated in a coordinated fashion by the same agonist, resulting in the transient

generation of distinct “waves” of ceramide increases that might serve to diversify the biological effects of ceramide. These studies also emphasize the significance of specific ceramide species in defined stages of cellular stress response.

For example, a transient increase of ceramide is observed within minutes after exposure to ionizing irradiation which is a consequence of DNA damage-independent acid SMase¹⁷³ or neutral SMase activation.⁴⁷ Several hours after irradiation, a second wave of ceramide accumulation is observed depending on the DNA damage-dependent activation of ceramide synthase. It seems that the late ceramide accumulation is also dependent on the first one and is rate limiting for the apoptotic process induced by irradiation.¹⁷³

Kroesen et al¹⁷⁴ on the other hand, showed that cross linking of the B-cell receptor generates C16-ceramide upstream of the mitochondria in a caspase independent manner and that inhibition of C16 ceramide generation rescues from cell death.⁵⁶ C24 ceramide however was generated downstream of mitochondrial dysfunction in a caspase dependent manner. All of the increases are seemingly due to activation of de novo synthesis and apparently different members of the ceramide synthase family might be differentially regulated.

A classical example of coordinated regulation of neutral and acidic SMase is the early work of Kronke and colleagues.¹⁷⁵ In these studies, stimulation with TNF α lead to activation of both enzymes through apparently separate mechanisms since different domains of the cytosolic tail of the TNF α receptor were responsible. Furthermore, while the activation of ASMase was linked to NF κ B activation, NSMase seemed to regulate the activation of proline-directed serine/threonine protein kinase(s).

Mechanisms of Ceramide Effects on Cellular Functions

Ceramide-Interacting Molecules

PKC ζ (reviewed in ref. 114)

PKC ζ , atypical PKC isoform, was identified as a molecule that ceramide directly binds and activate. Notably, ceramide-induced activation of PKC ζ is linked to inhibition of Akt-1. Akt-1 is a key regulatory molecule for various metabolic cellular functions, cell proliferation and cell death, which has been known for a long time to be inhibited by ceramide. Ceramide-activated PKC ζ seems to phosphorylate Ser³⁴ of the Akt-1 pleckstrin homology domain, thus preventing the interaction of Akt-1 with PIP₃ and respectively with the plasma membrane.¹⁷⁶⁻¹⁷⁸ In addition to stimulating the kinase activity of PKC ζ , ceramide binding seems to affect the ability of PKC ζ to interact with other proteins. In vascular smooth muscle, ceramide stabilizes the interaction of Akt-1 and PKC ζ within caveolin-enriched lipid microdomains to inactivate Akt and specifically reduces the association of PKC ζ with 14-3-3, a scaffold protein localized to less structured regions within membranes.¹⁷⁹ In differentiating embryonic stem cells, ceramide binding to PKC ζ similarly leads to activation of its kinase activity but also increases PKC ζ binding to its inhibitor protein, prostate apoptosis response-4 (PAR-4), thus compromising the antiapoptotic activity of PKC ζ and inducing apoptosis.¹⁸⁰

PP2A (reviewed in ref. 181)

Long chain *D-erythro*-C18-ceramide has been shown to activate PP2A (more specifically the AB³C trimer), PP2AC and PP1- γ C and - α C in vitro suggesting a direct and stereospecific effect of ceramide on the phosphatase activity. This ability of ceramide to activate PP2A was later found to mediate the ceramide effects on various substrates relevant to the induction of apoptosis, growth arrest or inflammation, including c-Jun, Bcl-2, Akt/PKB, Rb, PKC α , ERK1/2, IRAK-1, SR proteins and many others.

Cathepsin D

Ceramide specifically binds and activates the endosomal acidic aspartate protease cathepsin D. Direct interaction of ceramide with cathepsin D results in autocatalytic proteolysis of the 52 kDa procathepsin D to the enzymatically active 48/32 kDa isoforms that can subsequently

cleave and activate the apoptotic regulator Bid.¹⁸² Studies in ASMase deficient cells strongly suggest that ASMase activity is responsible for the generation of ceramide that can activate the protease.¹⁸³ This is in contrast to the ability of ceramide to activate PKC ζ and PP2A, which seemingly requires activation of the de novo pathway or NSMase.

Indirect Targets of Ceramide

Modulators of Apoptosis (reviewed in ref. 184)

The members of the Bcl-2 family of proteins are essential modulators of apoptotic cell death following genotoxic and nongenotoxic stress. Strong evidence links ceramide to the regulation of two members of that family, the antiapoptotic Bcl-2 and the pro-apoptotic Bax. The mechanisms involved are far from understood and apparently quite complex. TNF α - and etoposide-induced activation of NSMase leads to apoptosis via a pathway where ceramide is upstream of the antiapoptotic member of the family, Bcl-2 and lead to its inhibition (5).¹⁸⁵ These effects seem to involve PP2A. Bcl-2, whose phosphorylation at Ser⁷⁰ is required for its anti-apoptotic function, becomes dephosphorylated in response to ceramide in a PP2A-dependent manner and consequently is degraded in the proteasomes.¹⁸⁶ In turn, gemcitabine-induced ceramide generation is found to enhance the expression of proapoptotic Bcl-x.¹⁸⁷ Ceramide accumulation is also linked to the activation of the execution caspases, but it is downstream of the initiator caspases.^{188,189}

C16-ceramide, which is generated by ASMase in response to irradiation is shown to induce a conformation change of the pro-apoptotic member Bax leading to its activation and cytochrome C release.¹⁹⁰ It was suggested that only the production of ceramide in the mitochondria can induce the oligomerization of Bax that drives cell death.¹⁹¹ As Bcl-2, Bax is also regulated by ceramide via PP2A, since its dephosphorylation is associated with conformational change and release of cytochrome C from the mitochondria. Ceramide generation by ASMase has been suggested to mediate cell death by caspase- dependent and independent mechanisms depending on the death stimulus.¹⁹²

Regulators of Cell Cycle

Dephosphorylation of the Retinoblastoma protein, activation of the cyclin dependent kinase inhibitor p21 and inhibition of the cyclin dependent kinase 2^{193,194} are essential steps in the pathway leading to cell cycle arrest in response to ceramide accumulation. Ceramide induced-dephosphorylation of Retinoblastoma protein is mediated through PP2A, for which Retinoblastoma protein is a direct substrate. The hypophosphorylated Retinoblastoma protein binds and sequesters E2F, an essential factor for progression through the cell cycle. The senescent-associated growth may be attributed to a defect in the phospholipase D/protein kinase C (PLD/PKC) pathway and ceramide can inhibit both PLD and PKC.^{195,196}

Regulators of Inflammation

Studies by Hannun and Brenner were the first to show that TNF α activates the stress-activated protein kinases JNKs, resulting in the stimulation of AP-1-transcription factor and induces the translocation of NF κ B to the nucleus, resulting in the stimulation of NF κ B-dependent gene transcription, through ceramide.¹⁹⁷ Ceramide induced JNK activation has also been reported in response to FAS activation,¹⁹⁸ irradiation¹⁹⁹ and many cytokines. The activation of JNK by ceramide involves Rac-1²⁰⁰ in the case of radiation-induced apoptosis, PKC ζ in response to IL-1 β ²⁰¹ and TAK-1 in response to TNF- α , IL-1 β , or anti-Fas antibody.²⁰² In hepatocytes, nSMase2-generated ceramide has been shown to modulate the effects of IL-1 β on JNK phosphorylation and activity in PP2A and IRAK-1-dependent manner.¹³³

Ceramide-mediated JNK activation inhibits differentiation of skeletal muscle progenitor cells to myoblasts²⁰³ and induces the expression of various acute phase proteins like Insulin-like Growth Factor Binding Protein-1 in liver.²⁰⁴ JNK activation mediates apoptosis in neurons in response to ceramide and Amyloid β , the protein involved in the etiology of Alzheimer's disease. In that system, an inhibition of NSMase was found to attenuate Amyloid β -induced JNK phosphorylation and AP-1 DNA binding activity suggesting the involvement of the plasma membrane-associated SMase activity.²⁰⁵

Ceramide Effects on Membrane Organization

Some of the biological responses to ceramide generation are probably due to the effects ceramide has on the structure of membrane rafts and caveolae, on the membrane curvature and the membrane permeability to aqueous solutes. The two hydroxyl groups, the amid group protons and the trans-double bond in ceramide molecule, together with the lack of large hydrophilic head group determine the ability of ceramide to affect the properties and organization of biological membrane. The long-chain base and long saturated N-acyl chains accompanied by a very small hydrophilic head promote the partitioning of ceramide into ordered membrane domains. Together with SM, which has a strong affinity for interacting with membrane cholesterol, ceramide participate in the formation of the caveolae, the signaling platforms on the cell surface. While SM is by far much more abundant than ceramide in the lipid rafts, the generation of ceramide within rafts dramatically alters the biophysical properties of these membrane domains. Ceramide molecules have the tendency to spontaneously self associate to small ceramide-enriched membrane microdomains.²⁰⁶ Gulbins and colleagues had proposed that these ceramide microdomains spontaneously fuse to form large ceramide-enriched macrodomains or platforms. The formation of such platforms is proposed to underline the process of agonist induced receptor clustering and consequently, the reorganization of intracellular signaling molecules to transmit a signal into the cell. Among the receptors affected by ceramide-mediated aggregation/clustering are CD95, CD28, TNF α , CD40, Fc γ RII.²⁰⁷ Intriguingly, the acidic form of SMase seems to be involved in the formation of these signaling platforms, since it has been shown to translocate to the plasma membrane under certain conditions. In further support to that extend, overexpression of the SM synthase, SMS-1, which enriches plasma membrane with SM also enhances the translocation of Fas into lipid rafts leading to subsequent Fas clustering, DISC formation, activation of caspases and apoptosis, suggesting that SM levels in the lipid rafts might also be a critical factor possible by acting as a source for ceramide.²⁰⁸

Another intriguing property of ceramide is its ability to spontaneously form channels into planer bilayers, liposomes and biological membranes indicating that ceramide might regulate membrane leakiness. Experiments with unilamellar vesicles show that ceramide forms large pores that can allow the efflux of proteins as large as 60 kDa. Studies by Colombini and colleagues had shown that at physiologically relevant levels, ceramides form stable channels in mitochondrial outer membranes capable of passing the largest proteins known to exit mitochondria during apoptosis including cytochrome C.²⁰⁹ Ongoing efforts on characterizing the ceramide metabolism in this organelle will shed more light into the physiological function of ceramide in the mitochondria.

Finally, studies in cells isolated from ASMase-deficient mice or Niemann-Pick patients had shown defects in the phagolysosomal fusion and rab-4-mediated endocytosis²¹⁰ indicating that ASMase regulates select vesicular fusion processes by modifying the steric conformation of cellular membranes.²¹¹ These conclusions are supported by series of studies on the infectivity of *Listeria monocytogenes* and *Mycobacterium avium* discussed earlier.

Conclusion

It was realized long ago that sphingolipid structure is highly diverse and encodes distinct chemical information that can be conveyed during cell signaling.²¹² Research spanning through the last two decades established experimentally the role of this novel family of bioactive lipids in signaling. Ceramide emerged as the key metabolite in several sphingolipid-based signaling networks and was recognized to mediate conserved pathways of cellular stress response. With the genetic cloning and characterization of the majority of ceramide metabolizing enzymes, it also became clear that ceramide metabolism is finely tuned leading to spatial, temporal and species-specific accumulation during stress. A strict substrate specificity and distinct cellular localization of the enzymes that catalyze ceramide synthesis and degradation has brought diversity in the signaling molecules and cellular responses regulated by ceramide. The distinct ability of ceramide to alter the biophysical properties of cellular membrane adds additional layer of complexity in our understanding of the roles of ceramide in cellular stress response.

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