Preface

Regucalcin was discovered in the year 1978 as a calcium-binding protein that does not contain the EF-hand motif of the calcium-binding domain. Regucalcin plays a pivotal role in regulating intracellular calcium homeostasis and consequently has a profound effect on multiple intracellular signal transduction pathways.

The regucalcin gene is localized on the X chromosome in human and rats, and it is thought as a protein that is highly differentiated because of a great conservation of the regucalcin genes throughout evolution in vertebrates specifies. The regucalcin gene expression is regulated through many transcriptional factors (including AP-1, NF-A1, RGPR-p117, β-catenin, SP1, NF-κB and others). Regucalcin plays a multifunctional role in cellular regulation; a role in keeping intracellular Ca^{2+} homeostasis, an inhibitory role in protein kinases, protein phosphatases, NO synthase, nuclear deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis. Regucalcin suppresses an enhancement of cell proliferation and apoptotic cell death that are induced by various signaling factors. Regucalcin is the first time finding in protein molecule that has a role as a suppressor protein in cell signal transduction. Regucalcin is a key molecule in metabolic disease; its overexpression induces osteoporosis and hyperlipidemia. The deficiency of regucalcin is related to regulation of oxidative stress and to inducing of cell apoptosis. The downregulation of regucalcin gene expression is involved in the development of carcinogenesis in liver cells. Regucalcin plays a pathophysiological role in many metabolic disorders in human. This book introduces recent topics of regucalcin studies concerning the genomics, cell regulation and disease.

By the way, calcium concentration in the blood is controlled by a complex tuned mechanism. Serum calcium homeostasis is regulated through calcium-regulating hormones that act on the bone, kidney, liver, and intestine. The disturbance of serum calcium homeostasis affects the physiological functions of many cell and tissues.

The concentration of intracellular free calcium ion is $\sim 10^{-7}$ M, and its level is 10^4 fold lower than that of the extracellular fluid. The considerable gradient provides the potential for the influx of calcium ion (Ca^{2+}) into cells. A wide range of stimuli can lead to an increase in cytosolic Ca^{2+} concentration either from the extracellular space or from intracellular stores. The elevated cytosolic free Ca^{2+} (calcium signal) is transmitted to intracellular responses, which are modulated through a family of calcium sensitive proteins. Calcium signal plays an important role in the regulation of cell functions.

Generally, signal transduction plays a pivotal role in cellular regulation. The second messenger is generated in cells once the first messenger (hormone, cytokine, and neurotransmitter) binds to the receptors of the plasma membranes in cells. The second messenger, which was found firstly, is cyclic adenosine monophosphate (cyclic AMP). After that, the intracellular Ca^{2+} has been demonstrated to play a role as a second messenger for hormonal stimulation in cells. Calcium signal is partly transmitted to intracellular responses, which are modulated through a family of calcium-binding proteins, protein kinases, and protein phosphatases. The effect of calcium is amplified through these factors in cells. Calcium signal plays an important role in the regulation of many cell functions; muscle contraction, neurotransmission, hormone secretion, cell mitosis, differentiation, growth, chemotaxis cell motility, metabolism, gene expression, and others.

The author demonstrated that the liver plays a physiological role in the regulation of serum calcium metabolism through hepatic bile system in rats, and that hepatic bile calcium excretion is stimulated through hormonal stimulation. On the basis of this finding, the author reported the existence of a calcium-binding protein, which regulates the effect of Ca^{2+} in liver cells [Yamaguchi M and Yamamoto T: Binding of calcium by soluble fraction from normal rat liver [*Chemical and Pharmaceutical Bulletin*, 23: 2418-2421 (1975)]. Three years later, the author isolated a novel calcium-binding protein (regucalcin; RGN), which differs from calmodulin and other calcium-related proteins, from the hepatic cytoplasm of rats. This finding was in the year 1978.

The name of "regucalcin" was proposed for this calcium-binding protein that reverses the activities of various enzymes, which are activated or inhibited by Ca^{2+}- or Ca^{2+}/calmodulin in liver cells.

The regucalcin gene (*Rgn*) is found in many vertebrate and invertebrate species. Regucalcin protein and its gene are identified in over 15 species, which compose regucalcin family. The nucleotide sequences of regucalcin from vertebrate species are highly conserved in their coding region throughout evolution. After the finding of regucalcin, the identical protein to regucalcin was also reported as senescence marker protein-30 (SMP30) in the year1992.

The elucidation of the regulatory mechanism of the regucalcin gene expression is currently studied. The novel transcription factor RGPR-p117 was found by the author in the process of these studies. The expression of regucalcin mRNA is regulated by the administration of various hormonal factors *in vivo*. Regucalcin is greatly expressed in the liver and kidney cortex, and the protein is also found in other tissues and many cell types. The role of regucalcin in the regulation of cell functions in these tissues is investigated in detail.

There is growing evidence that regucalcin plays a multifunctional role as a regulatory protein in Ca^{2+}-dependent and -independent signaling processes in many cell types [reviewed in References. Regucalcin has been proposed to play a pivotal role in maintaining cell homeostasis and function as a regulatory (especially suppressor) protein of intracellular signaling systems.

This book introduces the findings concerning the genomics, cellular regulation and diseases that were found through regucalcin studies thus far, and it is composed from Chapter 1 to 14.

The structure of the regucalcin gene, transcriptional regulation of its gene, hormonal regulation of the gene expression, and the change in the gene expression in pathophysiological state are described in Chapter 1-4.

The regucalcin gene is located on the X chromosome in rat and human. The organization of the rat regucalcin gene consists of seven exons and six introns, and several consensus regulatory elements exist upstream of the 5'-flanking region (chapter 1). AP-1, NF1-A1, RGPR-p117, β-catenin and other factors have been found to be transcription factors in the enhancement of the regucalcin gene promoter activity (chapter 2). The transcription activity of the regucalcin gene is enhanced through intracellular signaling factors that are mediated through the phosphorylation and dephosphorylation of nuclear protein *in vitro*.

Regucalcin mRNA and its protein are markedly expressed in the liver and kidney cortex of rats. The expression of regucalcin mRNA in the liver and kidney cortex has been shown to stimulate through hormonal factors (including calcium, calcitonin, parathyroid hormone, insulin, estrogen, and dexamethasone) *in vivo* (chapter 3). Regucalcin mRNA expression is enhanced in the regenerating liver after partial hepatoectomy of rats *in vivo*.

The expression of regucalcin mRNA is suppressed in the liver and kidney under pathophysiological stress, suggesting an involvement of regucalcin in disease (chapter 4). Liver regucalcin expression is downregulated in tumor cells, suggesting a suppressive role in the development of carcinogenesis. Liver regucalcin is markedly increased into the serum of rats with chemically induced liver injury *in vivo*. Serum regucalcin has a potential sensitivity as a specific biochemical marker of chronic liver injury with hepatitis. Regucalcin has been proposed to be a key molecule in cellular regulation and metabolic disease.

Chapter 5-11 describes the recent topics of the role of regucalcin in cellular regulation, especially the role of regucalcin in intracellular calcium homeostasis (chapter 5), the reversible effect of regucalcin on calcium effect in enzyme regulation (chapter 6), the role of regucalcin in the regulation of nuclear signaling (chapter 7), the regulatory effect of regucalcin on protein synthesis and degradation (chapter 8), the suppressive effect of regucalcin on apoptosis mediated through cell signaling (chapter 9), the role of regucalcin as a suppressor in cell proliferation (chapter 10), and the role of regucalcin as a suppressor in liver regeneration (chapter 11).

Regucalcin plays a pivotal role in maintaining of intracellular Ca^{2+} homeostasis by activating Ca^{2+} pump enzymes in the plasma membrane (basolateral membrane), microsomes (endoplasmic reticulum), mitochondria and nuclei in many cell types. Regucalcin has a suppressive effect on calcium signaling from the cytoplasm to the nucleus in the proliferating cells. Regucalcin has also been demonstrated to transport to the nucleus, and it can inhibit the activities of Ca^{2+}-dependent protein kinase and protein phosphatase, Ca^{2+}-activated DNA fragmentation, and DNA and RNA synthesis in the nucleus. Regucalcin has been shown to have an inhibitory effect on protein synthesis and a stimulatory effect on protein degradation in liver cells.

Overexpression of regucalcin has been shown to inhibit the enhancement of cell proliferation due to hormonal stimulation. In addition, overexpression of regucalcin suppresses cell death and apoptosis in the cloned rat hepatoma cells induced by various signaling factors. Regucalcin has been demonstrated to play a pivotal role as a suppressor protein in the cell signaling system, and it is proposed that the protein plays a pivotal role in maintaining of cell homeostasis and function.

In the relation to disease, recent topic findings are introduced in Chapter 12-14. Animal models with overexpression and deficinency of regucalcin have been generated and the role of endogenous regucalcin *in vivo* has been shown. Bone loss has been found to induce in regucalcin transgenic (TG) rats with growth and aging (chapter 12). The mechanism is

resulted from the enhancement of osteoclastic bone resorption and the suppression of osteoblastic bone formation. Moreover, hyperlipidemia, hyperalbumina, and hypercalcemia have been seen in regucalcin TG rats with increasing age. The decrease in hepatic lipid and glycogen contents and the suppression of leptin and adiponectin expressions is also seen in the TG rats. Insulin resistance is caused by overexpression of regucalcin. The regucalcin TG rat model may be usefulness in determining the pathophysiological state and the development of therapeutic tools for metabolic disorder.

The deficiency of regucalcin (RGN/SMP30) induces a decrease in L-ascorbic acid (vitamin C) in mice, although this may not be significant because vitamin C is not synthesized *in vivo* in humans (chapter 13). Moreover, the recent findings of the pathophysiological role of regucalcin in relation to human diseases are introduced in chapter 14.

As described above, regucalcin has been proposed to be a novel-key molecule in cellular regulation and metabolic disease, and it may play an important role as a regulatory protein in maintaining the biological system in living organism.

The author believes that this book, which has been written to outline the recent advances of the novel protein regucalcin, will be of interest to scientists focused in the fields of biomedical research and life sciences.

Finally, the author would like to thank Nova Science Publishers, Inc., which has given the opportunity to publish this book.