CONCLUSION

Vitamin A and it derivatives (retinoids) exert profound effects on the regulation of cell growth and differentiation, mainly through two families of nuclear receptors, RARs and RXRs. These receptors are ligand-dependent transcription factors that bind to *cis*-acting DNA sequences, called RAREs and RXREs, located in the promoter region of their target genes. The RAR and RXR gene families are each composed of three subtypes, named α, β, and γ. To recognize a RARE, RARs usually must form a heterodimer with RXRs under physiological condition. However, not all RA-inducible genes contain RARE sequence(s) within their promoter.

In this thesis, I have described a novel mechanism for transcriptional regulation by retinoid through physical interaction between RAR/RXR and Sp1, and the expected biological consequences of this transcriptional regulation.

In the chapter I, I described how I reached to the finding of this novel mechanism. In the endothelial cells, RA up-regulates the expression of uPA and thereby plasmin levels. Elevation of PA/plasmin levels by RA causes the formation of active TGF-β, and this TGF-β mediates some biological actions of RA. Therefore, I have investigated the molecular mechanism by which RA stimulates the expression of uPA mRNA. I found the followings.: (1) In parallel with an increase in uPA mRNA retinoid induces RARs mainly via RARα. (2) Induction of uPA by RA is dependent upon RAR, and transactivation of the uPA promoter is enhanced by RAR/RXR. (3) Sp1 and its binding motif (GC box) within the uPA promoter are involved in the induction of uPA mRNA by RA. (4) RAR/RXR physically interact with Sp1 and potentiate its binding to GC box, resulting in a potentiation of uPA transcription.

Subsequently, in the chapter II, I analyzed the detailed molecular mechanism of this novel

interaction, especially as to whether RAR/RXR act as transactivator. I found that (1) RAR directly binds to C-terminus region of Sp1 containing the DNA-binding domain and that (2) RAR/RXR did not exhibit transactivation activity via physical interaction with the Sp1 fragment as a scaffold, suggesting that the enhancement of gene expression through RAR/RXR-Sp1 interaction is mainly dependent on potentiation of Sp1-derived transcription activity.

Collectively, I established that through physiological interaction with Sp1 RAR/RXR act as transcriptional modulator of Sp1 at least by potentiating its binding to the GC box. However, there remains the possibility that RAR/RXR also modulate Sp1's transactivation activity as coactivators.

Most recently, it is found that promoters of several other GC box containing genes known to be induced by RA in the endothelial cells can be transactivated through a similar mechanism (Shimada et al., unpublished observation). These genes include transglutaminase, TGF-β, and its signaling receptors. Namely, RA is revealed to enhance the expression of these genes via RAR-Sp1 interaction. In the latter two chapters, I have discussed about potential biological consequences of this novel mechanism. In the chapter III, I described that RA exhibits a potent anti-angiogenic activity in CAM and this effect is partially mediated by TGF-β. In the chapter IV, I described that RA exacerbates the progression of the hepatic fibrosis also via formation of TGF-β. During the pathogenesis of the liver fibrosis, a part of the stored vitamin A is converted to RA. In the HSCs, RA increases PA/plasmin levels probably via RAR/RXR-Sp1 interaction, and induces active TGF-β, leading to TGF-β-mediated super-induction of collagen and thus exacerbates the liver fibrosis.

Although I was able to show a possible link between transcriptional regulation via RAR/RXR-Sp1 interaction and these biological phenomena, the direct evidence remains to be proved. For

that purpose, I am planning to make dominant-negative mutants that specifically interfere the RAR/RXR-Sp1 interaction. These mutants might be useful tools to explore the current conclusion more directly. The experiments are now underway to map the amino acids responsible for the novel interaction.