

# Molecular Cell Biological Studies on the Regulation of Growth and Collagen Metabolism in Human Skin Fibroblasts by Vitamin C and Epidermal Growth Factor

Ryu-Ichiro Hata

*Medical Research Institute, Tokyo Medical and Dental University*

L-Ascorbic acid 2-phosphate (Asc2-P), a long-acting vitamin C derivative, stimulated transcription of genes for pro  $\alpha_1$  (I) and pro  $\alpha_2$  (I) collagen in normal human skin fibroblasts after 8 h of treatment in the absence or in the presence of cycloheximide, indicating Asc 2-P stimulates transcription of type I collagen genes in the absence of protein synthesis. The transcriptional rate in these cells reached the maximum value after 40 h of treatment, and at that time it was 3 to 4 times higher than that of the control cells cultured in the absence of Asc2-P. Steady state levels of mRNAs for pro  $\alpha_1$  (I) and pro  $\alpha_2$  (I) chains were also increased to be 3 to 4 times higher than the control levels by treatment of the cells with Asc2-P for 72 h. When the fibroblasts obtained from a patient with Ehlers-Danlos syndrome were treated with Asc2-P, the derivative also stimulated transcription of the gene for pro  $\alpha_1$  (I) chain and accumulation of mRNA for pro  $\alpha_1$  (I) chain. On the other hand, Asc2-P failed to stimulate transcription of the pro  $\alpha_2$  (I) gene or an increase in mRNA for pro  $\alpha_2$  (I) chain. Sodium ascorbate showed effects quite similar to those of Asc2-P, when fibroblasts obtained from a normal control or the patient were cultured for 16 h with it.

These results indicate the existence of cis-regulatory elements responsible for transcriptional activation by Asc2-P or ascorbic acid in pro  $\alpha_1$  (I) and pro  $\alpha_2$  (I) genes of normal fibroblasts. These data also suggest some defect (s) of these elements in the pro  $\alpha_2$  (I) gene of the patient with Ehlers-Danlos syndrome.