4. Expression of enamel protein by the methods for *in situ* hybridization and immunohistochemistry in the incisor of the amelogenesis imperfecta rat (ami)

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Mutation rat (ami) originated from SHR strain is characterized by amelogenesis imperfecta caused from the obvious reduction of the duration of the secretary stage of ameloblasts in tooth germ. In this report we observed the expression of mRNA for enamel protein in the incisor of ami by means of *in situ* hybridization and immunohistochemistry to elucidate the control mechanism of the quantity of enamel matrix formation.

5. Distribution and expression of bone sialoprotein (BSP) in the osteocartilaginous interface

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The present study used light and electron microscopic immunohistochemistry and *in situ* hybridization to examine the localization of bone sialoprotein (BSP) and its mRNA expression in epiphyseal growth plate and metaphyseal bone trabeculae of 10-day old rat tibia. Light and electron microscopic immunostaining for BSP and its mRNA expression was observed in hypertrophic chondrocytes, degenerative chondrocytes and osteoblasts at the osteocartilaginous interface. Furthermore, it was shown that, concomitant with the disruption of chondrocytes, BSP synthesized by degenerative chondrocytes was released extracellularly in the osteocartilaginous interface. These results indicate that BSP appear to have specific roles in bone formation and remodeling.

6. The localization and expression of bone sialoprotein (BSP) in developing rat mandibular bone

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The localization and expression of bone sialoprotein (BSP) and its mRNA were investigated in early bone formation of developing rat (embryonic day 14 -18) mandible using immunohistochemistry and *in situ* hybridization. The expression of BSP at gene and protein level was synchronous not only with the appearance of osteoblasts and their intercellular matrix in developing mandible at Day 15 but also with the appearance of the clusters of hydroxyapatite crystals at Day 16 -18. The observation of BSP in these critical stages of bone formation suggests that it may be involved in bone matrix mineralization.

7. Immunohistochemical study of transchondroid bone formation induced by BMP

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Previously we reported that the BMP-induced heterotopic bone was "chondroid bone", a tissue intermediate between cartilage and bone, by histochemical examinations. The newly formed bone tissue was formed by the course of "transchondroid bone formation", which was described by Yasui, 1997. Therefore we investigated immunohistochemical localization of typical matrix proteins of cartilage and bone. Both matrix proteins were detected in the matrix of "chondroid bone", in the early phase of BMP-induced heterotopic osteogenesis.

8. Type I, II, and X Collagen Gene Expression in Osteosclerotic (oc/oc) Mouse Femur

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Osteosclerotic (oc) mouse skeleton exhibits ricket-like lesion such as increases in osteoid and epiphyseal plate thickness, as well as osteosclerosis. To elucidate the pathogenesis of these skeletal disorders, we examined the expression of type I, II, and X collagen genes in the oc mouse femur by *in situ* hybridization. The expression of type II collagen mRNA was detected not only in epiphyseal cartilage bu in sclerotic diaphyses. The distribution appeared to well match in that of type I collagen, suggesting that the osteoblasts in oc mouse diaphyses coexpressed type I and II collagen genes.

9. The passaged bone marrow cells lose the activity of differentiation to osteoblasts

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