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STUDIES ON THE MECHANISMS OF PIGMENT PATTERN FORMATION AND LIMB PATTERN FORMATION

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To analyze cellylar mechanisms of pattern formation, we have studied amphibian pigment pattern and avian limb cartilage pattern, especially in cell culture. The pigment pattern of the amphibian body surface is specified by the distribution of three types of chromatophores. These chromatophores are of neural crest origin and differentiate depending on the environmental prepattern of the skin. The cartilage pattern of avian limb is specified in the distal of the limb bud, progress zone (PZ) under the control of the AER and ZPA.

We have succeeded in the clonal culture of three types of chromatophores isolated from bullfrog tadpoles. The proliferating melanophores retained the activity of melanin synthesis and melanin dispersion in clonal culture. However, iridophores and xanthophores transdifferentiated into melanophores in clonal culture, although *in vivo,* these chromatophores proliferated without the transdifferentiation. Thus, some factors must be operating to stabilize the differentiated states of these chromatophores involved in pigment pattern formation. We have cultured the iridophores in the medium containing the serum of bullfrog tadpoles. In this medium, iridophores proliferated without the conversion. One of such factors, melanization inhibiting factor (MIF), has been isolated later from the skin of *Xenopus laevis*. The MIF inhibited the differentiation of the melanophores and was rich in the ventral skin, suggesting some roles in the dorsoventral pigment pattern formation.

The cartilage pattern of chick limb bud is specified in the PZ, where the cells are maintained in an undifferentiated state by factors from the AER. We have found that FGF-2 (basic fibroblast growth factor) is one of the factors and induces the expression of MSX-1 and AV-1, which are markers of the progress zone, in the cultured PZ cells. The proliferation and chondrogenesis of the PZ cells were stimulated by retinoic acid (RA) in culture as in the case of RA-induced duplicate formation *in vivo*.

A positional heterogeneity of interaction was found in cultured limb bud fragments and mesenchymal cells. The cells of anterior margin responded to the signal from the posterior margin (ZPA) and proliferated. This type of interaction seems to be involved in the regulation of pattern in limb development.

The positional values along the anteroposterior and proximodistal axes of the limb bud are allotted to the PZ cells isolated from different parts of the limb bud must have different characteristics which are specified by the positional values. We have segregate from each other. This difference in cell affinity reflected the positional values specified in the PZ and was retained in the proximal tissues. Further, the cell affinity changed by the RA-treatment; posteriorization and proximalization on the cell surface characteristics occurred as *in vivo*.

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