STUDIES ON THE DIFFERENTIATION OF CARTILAGE

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Hyaline cartilage is characterized by the presence of chondroitin sulfate proteoglycan (CSPG), Type II collagen $[\alpha 1(II)]_3$ and link proteins. The core protein of CSPG and $[\alpha 1(II)]_3$ appear to be specific gene products whose synthesis is greatly enhanced during cartilage differentiation. The complete CSPG molecule is formed through post-translational modification of the core protein to CSPG as a result of the action of a series of glycosyl and sulfotransferases probably organized as a multi-enzyme complex.

When stage 24 chick limb bud cells are cultured at high density or over agar and subsequently at low density, differentiation to cartilage is observed. This differentiation may be monibored by the formation of metachromatic matrix, increased synthesis of proteoglycan or appearance of Type II collagen. If, however, limb bud mesenchyme is exposed to the thymidine analogue, 5-bromo-2'-deoxyuridine (BrdUrd) during the first 48 hours of culture, differentiation is irreversibly blocked.

In order to study this problem further, the following two lines of investigation have been pursued: (1) the elucidation of the cell-free synthesis of core protein of CSPG, and (2) a study of the mechanism by which incorporation of BrdUrd into the genome interferes with differentiation.

In order to pursue the first line, a method for the radioimmune assay of core protein has been developed. The method is based on the assumption that the CSPG subunit may be identified by labeling with $^{35}SO_4$. CSPG subunit, obtained from epiphyses of 13-day chick embryo tibia and femurs, was treated with highly purified testicular hyaluronidase to prepare an antigen for immunization of rabbits. The antisera obtained were assayed against a $^{35}SO_4$-labeled antigen derived from differentiated limb bud cultures. Although testicular hyaluronidase removes approximately 90% of the $^{35}SO_4$ counts of the subunit, the remaining antigen is sufficiently radioactive to be used in a radioimmune assay. An inhibition assay permits the quantitation of non-labeled core protein.

Utilizing antisera to core protein it has also been possible to demonstrate nascent core protein chains on polysomes prepared from differentiated limb bud cultures. The elongation of nascent chains on chick polysomes in a wheat germ system as well as <u>de novo</u> synthesis of immunoprecipitable protein directed by <u>RNA</u> extracted from differentiated limb bud cultures has been demonstrated.

Studies undertaken to define the mechanism by which BrdUrd interferes with differentiation indicated a difference between the incorporation of [^{3}H]thymidine and of [^{3}H]BrdUrd. When limb bud mesenchyme was exposed to [^{3}H]thymidine during days 1

and 2 of culture, the radioactivity was incorporated into repetitive, moderately-repetitive and unique classes of DNA, whereas [^{3}H]BrdUrd was preferentially incorporated into a moderately-repetitive region.

In order to further define the DNA fraction into which preferential incorporation had occurred, a [^{3}H]BrdUrd probe was prepared from chick limb bud mesenchyme. Non-radioactive DNA was prepared from embryonic liver, undifferentiated limb bud mesenchyme, embryonic sternal cartilage, differentiated limb bud cultures and BrdUrd-blocked cultures. Sheared non-radioactive DNA's were used in 100-fold excess to drive reassociation of sheared [^{3}H]BrdUrd probe. The purified mature cartilage DNA's of embryonic sternae and differentiated limb bud cultures drove the reassociation of the probe approximately two times faster than DNA from liver, undifferentiated limb bud or BrdUrd-blocked cells.

Calculations indicate that 10% of the thymidine residues in the total probe were substituted by BrdUrd. Density gradient fractionation demonstrated that total probe was made of a variety of fractions varying from 4% to 32% substitution. Kinetic analyses revealed that the most highly substituted fractions showed a maximum difference between reassociation with DNA's obtained from chondrocytes and DNA's obtained from prechondrocyte mesenchyme.

The results obtained indicate that the incorporated BrdUrd is clustered in the genome and such clusters are amplified during cartilage differentiation.

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