PROTEOGLYCANS SYNTHESIZED BY CHICK LIMB BUD CHONDROCYTES GROWN IN VITRO, Vincent C. Hascall, Ph.D., National Institute of Dental Research, Bethesda, Maryland 20014

Cartilage proteoglycans are polydisperse in molecular size, molecular weight and chemical composition.[1] Several parameters are known to contribute to the polydispersity. These include: (a) different numbers of glycosaminoglycan chains per core protein; (b) differences in the length of the glycosaminoglycan chains; (c) differences in the length of the core protein; (d) differences in the proportion of chondroitin sulfate and keratan sulfate; (e) differences in the proportion of chondroitin-4-sulfate and chondroitin-6-sulfate.[2-6] Many of these parameters can be studied in the chick limb bud culture system.[7] Mesenchymal cells from stage 23-24 chick limb buds are grown in vitro under conditions where a large proportion of the cells differentiate into chondrocytes by day 3. From day 4 to day 8 in culture, the chondrocytes mature, form nodules and elaborate an extensive extracellular matrix containing type II collagen[8] and proteoglycans characteristic of cartilage.[9] The proteoglycans synthesized by the mature, day 8 chondrocytes have the following properties[9,10]: (a) the monomers (S_o=19 Svedbergs) contain about 7% keratan sulfate and 85% chondroitin sulfate; (b) the keratan sulfate is located primarily in the keratan sulfate-enriched region of the core protein[2]; (c) 60-70% of the molecules interact with hyaluronic acid and form aggregates ($S_o \cong$120 Svedbergs); (d) the aggregates contain the link protein. The proteoglycans synthesized by the maturing chondrocytes (days 3-6) have some distinct differences. Most notable are a gradual decrease in the proportion of chondroitin-6-sulfate from 70% (day 3-4) to about 55% (day 9-10) and a gradual increase in the proportion and size of the keratan sulfate chains. Similar changes have been noted for other cartilages during development.[6] The proteoglycans synthesized in older cultures (day 16-24) gradually decreased in molecular size, as observed by elution profiles on Sepharose 2B, but the proportion of molecules capable of binding to hyaluronic acid remained the same as for day 8 proteoglycans. In an experiment designed to investigate the molecular parameters relating to these changes,[11] proteoglycans were isolated from a culture that was labeled on day 8 with [^{3}H]-serine and, subsequently, on day 16 with [^{14}C]-serine. Analyses of these proteoglycans indicated: (a) although 40-50% of the molecules synthesized on day 8 have been turned over,[10] those still present in the matrix of the day 16 culture were of the same size as those synthesized and isolated directly from day 8 cultures; (b) the proteoglycans synthesized by the same cells on day 16 were significantly smaller on Sepharose 2B; (c) the smaller size was related, in part, to a decrease in the average size of the chondroitin sulfate chains synthesized on day 16. Electron microscopy[12] was used to visualize aggregates isolated from day 8 and day 16 cultures as well as from the epiphyses of 13-day old chicks.[11] The size distributions of the lengths of the monomers bound to aggregates were identical and Gaussian for the day 8 and epiphyseal preparations (312 nm $\pm$ 62 nm). For comparison, monomers in bovine nasal aggregates exhibited a Gaussian distribution with a much larger mean and standard deviation (343 nm $\pm$ 95 nm). The size distribution for monomers in aggregates from the day 16 cultures was similar to that for the day 8 monomers but significantly shorter (297 nm $\pm$

58 nm), suggesting that shorter average core lengths may also contribute to the decreased average molecular size observed for proteoglycans synthesized in the older cultures. Such molecular changes may also be important in the alterations in proteoglycans which occur during cartilage aging.[6] The biosynthesis of proteoglycans in day 8 cultures was studied using [^{3}H]-serine and [^{35}S]-sulfate with a 10 min. pulse followed by different chase times.[13] While the ratio of [^{3}H]-serine to [^{35}S]-sulfate in monomer proteoglycans increased from the 0 min. through 25 min. chase times, both precursors were uniformly present in the entire size distribution of monomers observed on Sepharose 2B at all chase times, suggesting that the inherent polydispersity of the proteoglycans reflects biosynthetic processes. The relevance of the proteoglycan polydispersity for cartilage function remains to be determined as do many of the biosynthetic mechanisms, translational and post-translational, that are involved in generating such variation. The culture system described in this report should provide a useful model for further study of these problems.

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