

Alteration of Proteoglycan Metabolism during the Differentiation of 3T3-L1 Fibroblasts into Adipocytes

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Abstract. 3T3-L1 fibroblasts were induced to differentiate to 3T3-L1 adipocytes by dexamethasone, isobutylmethylxanthine, and insulin. To study how differentiation affects extracellular matrix production, the accumulation of proteoglycans was studied by labeling the 3T3-L1 cells with [³⁵S]sulphate for 24 h. The labeled proteoglycans were isolated from the medium and cell layer extracts by anion-exchange chromatography. They were then taken to gel filtration chromatography on Superose 6 before or after chondroitin ABC lyase digestion. Hyaluronan was determined by radioimmunoassay.

The rate of accumulation of proteoglycans and hyaluronan in the control 3T3-L1 fibroblasts increased with time whereas it decreased slightly in the age matched adipocytes where the differentiation had proceeded, as

judged by the change of morphology and increase of the activity of the adipose conversion markers glycerol-3-phosphate dehydrogenase and hormone sensitive lipase. The main change noted was that the adipocytes accumulated 50–70% less amount of small proteoglycans (decorin) in the medium than the fibroblasts did. The amount of large chondroitin/dermatan sulphate proteoglycans was also decreased but to a considerably smaller extent (30%). In the cell layer, heparan sulphate proteoglycan decreased by 60% as compared with the control cells.

Thus, the differentiation of 3T3-L1 fibroblasts into adipocytes, which changes the morphology and the function of the cells, is also accompanied by a decreased net production especially of proteoglycans typical of fibrous connective tissue.

THE 3T3-L1 fibroblast cell line is derived from mouse embryo. The cells are similar to other 3T3 cells regarding the synthesis and secretion of collagen and hyaluronan (Green and Meuth, 1974). Once 3T3-L1 fibroblast cultures become confluent, the proliferation is arrested and the cells can be differentiated into adipocytes under certain conditions (Green and Kehinde, 1974, 1976). The changes in cell morphology and the accumulation of droplets of triglycerides are accompanied by increased activities of enzymes participating in fat metabolism; both lipogenic (Pairault and Green, 1979) and lipolytic (Grimaldi et al., 1978).

The adipose cells also show an increase in the amount of insulin receptors and sensitivity to hormones (Rubin et al., 1987). Other changes associated with the adipose conversion are the decreased expression of actin and tubulin (Spiegelman and Farmer, 1982) and the enhanced synthesis and secretion of type IV collagen and entactin (Aratani and Kitagawa, 1988). Changes in the transcription of adipose specific, not yet identified, mRNA as a result of differentiation have also been reported (Bernlohr et al., 1984).

This study was undertaken to investigate if there is any relation between a changed phenotype of 3T3-L1 cells and their ability to synthesize and degrade extracellular matrix components and if these components play any role in the differentiation process.

Materials and Methods

Materials

DME and sera were obtained from Gibco, Grand Island, NY. Dexamethasone, isobutylmethylxanthine, guanidine hydrochloride (practical grade), and urea were purchased from Sigma Chemical Co., St. Louis, MO, and porcine insulin was from Novo, BioLabs, Denmark. Solutions of guanidine hydrochloride were filtered through activated charcoal, while stock solutions of urea were passed through a bed of mixed ion-exchange resin before use. Chondroitin ABC lyase was purchased from Miles Laboratories, Inc., Naperville, IL and [³⁵S]sulphate was a product of Amersham Corp., Arlington Heights, IL. DEAE-cellulose, DE 52, was from Whatman Inc., Clifton, NJ. The HA-50 kit with ¹²⁵I-hyaluronan binding protein and the Superose 6 HR/50 column were purchased from Pharmacia, Uppsala, Sweden. The SDS-PAGE molecular weight standard designated HMW was bought from Bio-Rad Laboratories, Richmond, CA.

Cell Culture

3T3-L1 fibroblasts were grown until confluence was reached in 35-mm dishes in DME with 10% newborn bovine serum. After that, the cells in some of the dishes were differentiated while the remaining cells were kept as fibroblast control cells in DME with 10% FBS (standard medium). The differentiation into adipocytes was initiated by the addition of standard medium supplemented with 0.25 μM dexamethasone, 0.5 mM isobutylmethylxanthine, and 2 μg/ml of insulin (Ronnelt et al., 1982). After 2 d the medium was replaced with standard medium with insulin (2 μg/ml) added to it. After another 2 d the cells received fresh standard medium which was

then replaced three times a week. Judged by microscopy, 70–80% of the cells were converted to adipocytes after 10 d.

Extraction and Isolation of Proteoglycans

The adipocytes and the fibroblasts, which were kept in culture without passage for the same length of time as the adipocytes, received fresh standard medium containing 100 $\mu\text{Ci}/\text{well}$ of [^{35}S]sulphate and after 24 h the medium was removed. The cell layer was rinsed twice with 1 ml of cold PBS, pH 7.4 (137 mM NaCl, 2.68 mM KCl, 1.47 mM K_2PO_4 , and 1.12 mM NaH_2PO_4) and this material was pooled together with the removed medium and kept frozen until further analysis. The remaining cell layer was extracted overnight at 4°C with 1.5 ml of 4 M guanidine hydrochloride, 50 mM acetate pH 5.8, 10 mM EDTA, 5 mM *N*-ethylmaleimide, 10 mM ϵ -amino-*n*-caproic acid, 5 mM benzamidine, and 1% Triton X-100. The extract of the cell layer was diluted with 25 vol of 6 M urea, 10 mM EDTA, 50 mM acetate pH 5.8, 5 $\mu\text{g}/\text{ml}$ ovalbumin, and 0.1% Triton X-100 before purification. The removed medium and the cell layer extract were then subjected to ion-exchange chromatography on DEAE cellulose columns (0.8 \times 1 cm) (Carlstedt et al., 1981). The columns were washed with 6 M urea and 50 mM acetate pH 5.8 buffer until the free [^{35}S]sulphate had been washed away. Hyaluronan was eluted with 5 ml of 6 M urea in 0.5 M acetate pH 5.8. The proteoglycans were eluted with 3 ml of 4 M guanidine hydrochloride and 50 mM acetate pH 5.8 buffer. All buffers used for separation of the components from the cell layer extract contained 0.1% Triton X-100.

Characterization of Proteoglycans

The different classes of proteoglycans were separated by applying aliquots of the DE 52 proteoglycan fraction supplemented with carrier proteoglycans (Cöster and Fransson, 1981) to the Superose 6 HR/50 column which was operated at a flow rate of 0.25 ml/min with 4M guanidine hydrochloride, 50 mM acetate, pH 5.8, and 0.1% Triton X-100.

Degradation Methods

To determine the amount of heparan sulfate proteoglycans, aliquots of DE 52 proteoglycan samples were first dialyzed against 5 \times 200 ml of 0.1 M Tris-acetate, pH 7.3, 10 mM EDTA and 0.1% Triton X-100. They were then digested with 15 mU of chondroitin ABC lyase at 37°C for 4 h and later subjected to chromatography on Superose 6. Before dialysis, carrier proteoglycans were added and the proteoglycans were protected against proteolysis by the addition of 10 μg of ovomucoid.

Analytical Methods

Aliquots of hyaluronan fractions from the DE-52 columns were taken in order to determine the amount of hyaluronan using the [^{125}I]-hyaluronan binding protein kit.

For the purpose of determining the protein concentration, the glycerol-3-phosphate dehydrogenase (GPDH) and the hormone sensitive lipase (HSL) activity, the cells were washed twice with PBS, pH 7.4. They were then scraped off in 0.25 M sucrose, 10 mM Tris-HCl pH 7.4, 1 mM EDTA, 1 mM dithiothreitol, homogenized in a Potter-Elvehjem homogenizer and stored at -70°C until analysis was performed. Protein was determined according to Bradford (1976) with BSA as standard. The GPDH and HSL activities in the cell homogenates were determined according to Ronnett et al. (1982) and Fredrikson et al. (1981), respectively, and expressed as milliunits per milligram of protein. The HSL activity was inhibited by 100 mM NaF by 92, 94, and 94% in cells harvested 5, 8, and 15 d, respectively, after the induction of differentiation (Fredrikson et al., 1981).

^{35}S was counted in an LKB-Wallach scintillator counter with automatic quench correction and [^{125}I] in an LKB-Wallach gammacounter.

Proteoglycan samples were prepared for gel electrophoresis by precipitating the proteoglycan containing samples with 3 vol of ethanol at 4°C. The pellet was washed once with 2 ml ethanol, then dissolved in SDS-PAGE sample buffer (Laemmli, 1970), and subjected to electrophoresis on a 3–12% gradient SDS-polyacrylamide slab gel (Fisher et al., 1989). After fixing, staining, and soaking the gel in 1.3 M salicylate and drying it on a slab gel dryer, autoradiography was performed with Kodak XAR film at -70°C until the desired intensity was achieved.

1. *Abbreviations used in this paper:* GPDH, glycerol-3-phosphate dehydrogenase; HSL, hormone sensitive lipase.

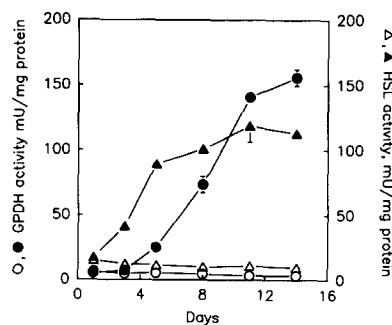


Figure 1. Time dependence of GPDH and HSL activity in confluent 3T3-L1 cell cultures. Cell extracts were prepared at the indicated time after confluence as described in Materials and Methods and tested for GPDH and HSL activity. (Open symbols) Nondifferentiated 3T3-L1 fibroblasts; (filled symbols) 3T3-L1 cells subjected to induced adipose conversion.

Results

Extent of Differentiation

The adipose conversion of the 3T3-L1 fibroblasts was visible as the accumulation of fat droplets in the cells and the increased activity of GPDH and HSL, which serve as differentiation markers (Fig. 1). Starting on the fifth day after the beginning of the induction of the differentiation, the activity of GPDH, a lipogenic enzyme, increased steadily in the differentiated adipocytes to reach a plateau between day 11 and 14. The activity of HSL started to increase on the second day and reached the plateau on the eighth day. The control 3T3-L1 fibroblasts did not show any increase in the activity of these enzymes during the culture period of 2 wk (Fig. 1). The activity of GPDH increased ~ 45 -fold while the activity of HSL increased 12 times, as the conversion from fibroblasts to adipocytes proceeded.

The protein content in the fibroblast and adipocyte monolayers increased by 60 and 70%, respectively, during the 2 wk of culture after confluence was reached.

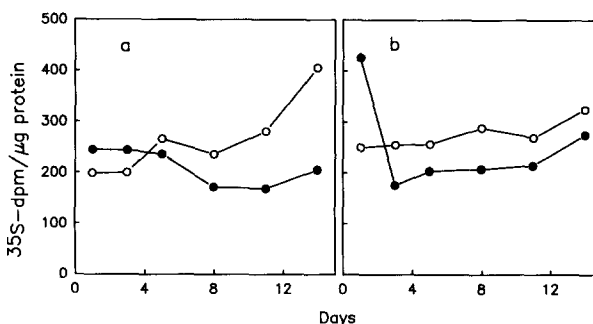


Figure 2. Diurnal incorporation of [^{35}S]sulphate into the proteoglycan fraction of 3T3-L1 cells at various times after confluence. 3T3-L1 cells were labeled with [^{35}S]sulphate for 24 h at various times after confluence. (a) Proteoglycans secreted into the medium and (b) proteoglycans associated with the cell layer. Open symbols represent results with undifferentiated 3T3-L1 fibroblasts and filled symbols represent 3T3-L1 cells subjected to induced adipose conversion as described in Materials and Methods. The time scale refers to the end of the labeling period.

Relation between Differentiation and Proteoglycan and Hyaluronan Metabolism

To investigate if the net rate of incorporation of [35 S]sulphate into proteoglycans changes during the differentiation of 3T3-L1 fibroblasts into adipocytes, the cells were labeled with [35 S]sulphate for 24 h at different stages of conversion. The rate of accumulation of proteoglycans in the medium, expressed as dpm/ μ g protein, increased steadily with time in the 3T3-L1 fibroblast control cultures (Fig. 2 a). 14 d after confluence, the net rate of [35 S]sulphate incorporation expressed as dpm/ μ g protein was elevated by \sim 100%. In the 3T3-L1 adipocytes the differentiation inducers stimulated the incorporation of the labeled compound into the proteoglycans secreted to the medium during the first 3 d of adipose conversion. As the differentiation inducers were withdrawn and the accumulation of fat droplets became visible, the net rate of incorporation of the labeled sulphate started to decrease on the fifth day after the start of the conversion to reach a level slightly lower than the initial level found in the media of 3T3-L1 fibroblasts on the first day after confluence (Fig. 2 a). When comparing the fibroblasts and adipocytes 14 d after confluence, the 3T3-L1 adipocytes incorporated 50–60% less of [35 S]sulphate into the proteoglycans secreted into the medium than the 3T3-L1 fibroblasts did (Fig. 2 a).

With the exception of the induction period for the adipocytes, the net rate of incorporation of [35 S]sulphate into the cell layer associated proteoglycans was almost constant with time in both the fibroblasts and the adipocytes cultured under the same conditions (Fig. 2 b). However, the adipocytes showed 20–60% lower net rate of incorporation of [35 S]sulphate into the cell layer-associated proteoglycans. The variability was dependent on the cell batch used.

The diurnal net rate of hyaluronan secretion to the medium by 3T3-L1 fibroblasts increased 2.5-fold during the 2 wk of culture that took place after confluence was reached (Fig. 3 a). In the adipocytes the amount found in the medium 24 h after the start of adipocyte conversion was 60% lower than that of the corresponding control fibroblasts and the amount found in the medium remained constant during the following

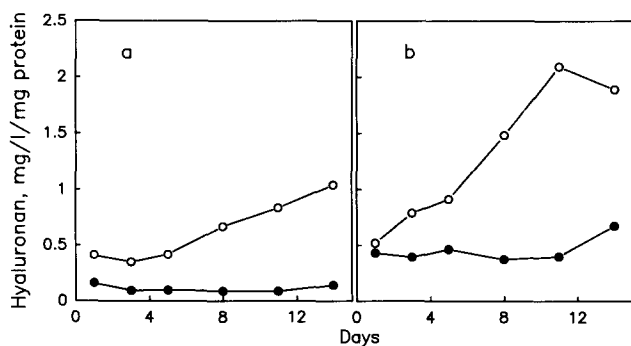


Figure 3. The amount of hyaluronan produced by 3T3-L1 cells at various times after confluence. The cells received fresh medium 24 h before the sampling as described in Materials and Methods. (a) Hyaluronan secreted into the medium and (b) hyaluronan associated with the cell layer. Open symbols represent 3T3-L1 fibroblasts and filled symbols represent 3T3-L1 cells subjected to induced adipose conversion as described in Materials and Methods. The time scale refers to the time of sampling.

2 wk (Fig. 3 a). The diurnal net synthesis of adipocyte hyaluronan associated with the cell layer also remained approximately constant during that period (Fig. 3 b). The amount found 24 h after the start of adipocyte conversion was the same as for the corresponding fibroblasts. However, the diurnal net accumulation of hyaluronan increased more than three times in the cell associated layer of the control fibroblasts over the 2-wk period (Fig. 3 b).

Characterization of Proteoglycans

To characterize further the differences between the proteoglycans produced by the 3T3-L1 adipocytes and the corresponding 3T3-L1 fibroblasts, the size distribution and the chondroitin ABC lyase resistance (i.e., the relative proportion of chondroitin/dermatan sulphate to heparan sulphate proteoglycans) was analyzed. Proteoglycans from the media of cells left for 14 d after confluence were subjected to gel chromatography on Superose 6. It was found that the 35 S activity of the large proteoglycans (Fig. 4 a, fractions 17–23) in the adipocyte samples was 30% lower than in the fibroblast samples, measured as dpm/ μ g protein. For the small proteoglycans (Fig. 4 a, fractions 24–35) the relative difference was even larger, in that the specific radioactivity (dpm/ μ g of protein) was 60% lower in the adipocyte media (Fig. 4 a).

For further characterization proteoglycan samples were subjected to digestion with chondroitin ABC lyase in order to degrade chondroitin and dermatan sulphate chains. Chromatography on Superose 6 revealed that only 8% of the proteoglycan bound 35 S activity from the media remained as heparan sulphate proteoglycans, which chromatographed on Superose 6 at a position intermediary to the large and small proteoglycans (fractions 17–35, Fig. 4 b) (Schmidtchen et al., 1990).

Proteoglycans from the cell layer of the fibroblasts and adipocytes showed a polydisperse pattern in the Superose 6

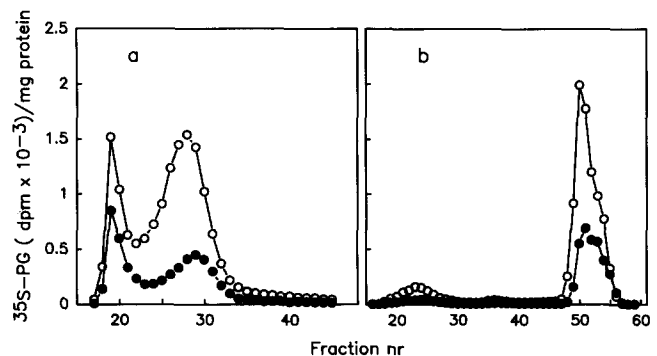


Figure 4. The Superose 6 chromatogram of proteoglycans found in the media of 3T3-L1 cells before and after chondroitin ABC lyase treatment. 2 wk after confluence, 3T3-L1 cells were labeled for 24 h with [35 S]sulphate and the medium proteoglycans were prepared on DEAE cellulose as described in Materials and Methods. Proteoglycan samples were then chromatographed on Superose 6 without (a) or after prior chondroitin ABC lyase digestion (b). Open symbols represent 3T3-L1 fibroblasts and filled symbols represent 3T3-L1 adipocytes obtained as described in Materials and Methods.

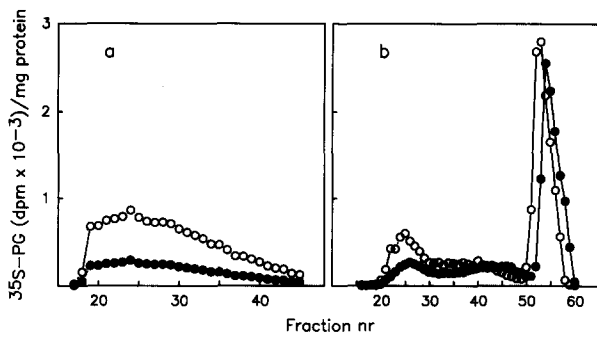


Figure 5. The Superose 6 chromatogram of proteoglycans found in the cell layer of 3T3-L1 cells before and after chondroitin ABC lyase treatment. 2 wk after confluence, 3T3-L1 cells were labeled for 24 h with [³⁵S]sulphate and the cell layer proteoglycans were prepared on DEAE cellulose as described in Materials and Methods. Proteoglycan samples were then chromatographed on Superose 6 without (a) or after prior chondroitin ABC lyase digestion (b). Open symbols represent 3T3-L1 fibroblasts and filled symbols represent 3T3-L1 adipocytes obtained as described in Materials and Methods.

chromatograms (Fig. 5 a). No distinct peaks were found in the fractions corresponding to the large and small proteoglycans found in the media, neither in the fibroblasts, nor in the adipocytes. However, the amount of ³⁵S radioactivity found

in fractions 17–35 was ~70% lower in the adipocytes than in the control fibroblasts. Chondroitin ABC lyase digestion followed by separation on Superose 6 showed that only 24 and 7% of the radioactivity of the fibroblast and adipocyte samples, respectively, was recovered in fractions 17–35, indicating the presence of heparan sulphate proteoglycans. The component peaked in fraction 24 in the fibroblast samples (Fig. 5 b).

The autoradiogram after SDS-PAGE of the pooled fractions 17–23, corresponding to large proteoglycans from the medium, revealed the presence of proteoglycans with an apparent relative molecular mass well above 200 kD relative to the protein standard (extrapolation of a diagram of the log relative molecular mass of the standard proteins versus the run distance yields the value of 600 kD) in the media of both adipocytes and fibroblasts (Fig. 6 a, lanes a and c). The small proteoglycans found in the pooled fractions 24–35 corresponded to an apparent *M_r* of 140 and 230 kD, respectively (Fig. 6 a, lanes b and d). The bands representing these two species from the media of the adipocytes were not as strong as those from the control fibroblast media. The dominating small proteoglycans of both fibroblasts and adipocytes appeared to be of the PG-S2 type (Fischer et al., 1989). The autoradiogram of the gel after SDS-PAGE of both the pooled fractions 17–23 and 24–35 from the Superose 6 chromatography of the proteoglycans from the cell

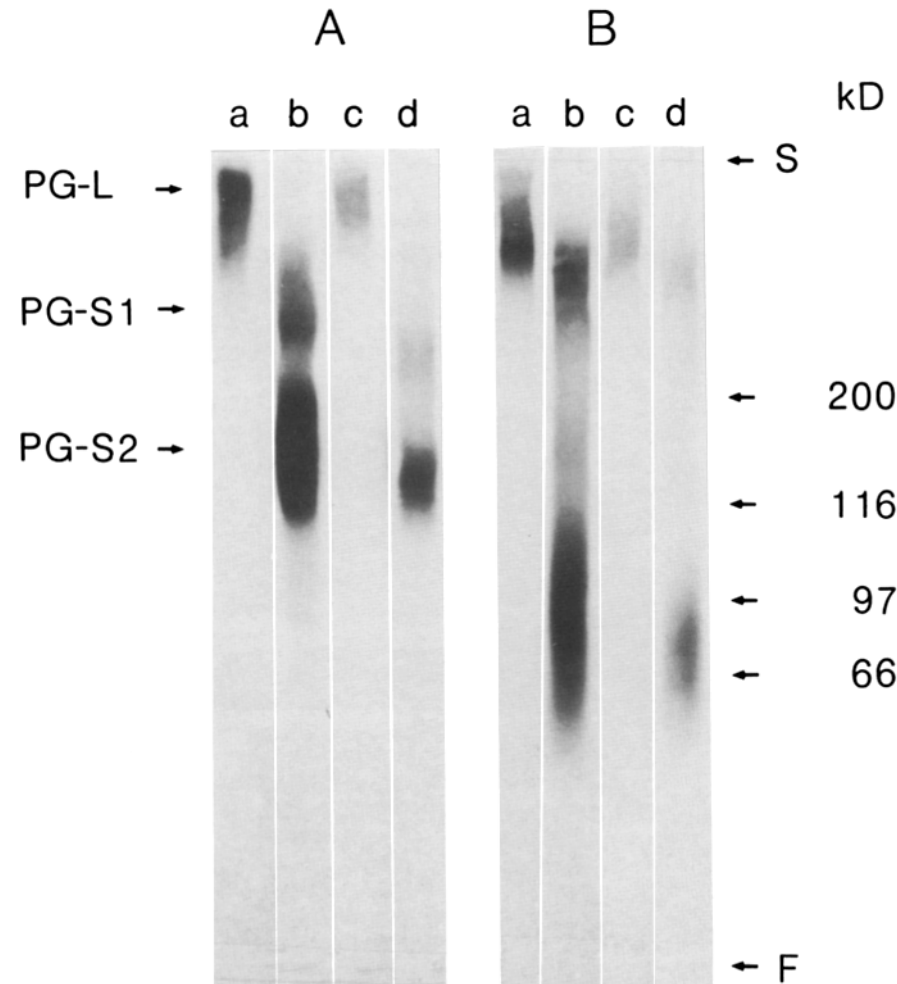


Figure 6. Autoradiogram of pooled Superose 6 fractions of [³⁵S]sulphate labeled proteoglycans from 3T3-L1 cells. 2 wk after confluence, 3T3-L1 cells were labeled for 24 h with [³⁵S]sulphate and the proteoglycans were prepared on DEAE cellulose and separated on Superose 6 (see Figs. 4 a and 5 a) as described in Materials and Methods. Fractions 17–23 and 24–35, respectively, were pooled and samples analyzed by SDS-PAGE on a 3–12% gel. A shows medium proteoglycans (corresponding to the chromatograms in Fig. 4 a) and B shows cell layer proteoglycans (corresponding to the chromatograms in Fig. 5 a) from 3T3-L1 fibroblasts (lanes a and b) and adipocytes (lanes c and d). Lanes a and c refer to the pooled fractions 17–23 while b and d refer to the pooled fractions 24–40. S indicates the start and F indicates the front. The positions of the proteins of the molecular weight standard are also shown.

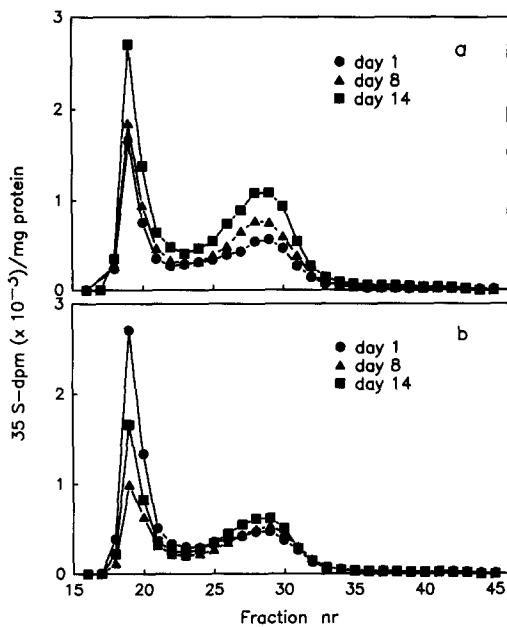


Figure 7. The Superose 6 chromatograms of proteoglycans found in the media of 3T3-L1 cells at various times after confluence. 3T3-L1 cells were labeled for 24 h with [³⁵S]sulphate and the medium proteoglycans were prepared on DEAE cellulose as described in Materials and Methods. Proteoglycan samples were then chromatographed on Superose 6. The media were collected from the cultures 1 (circles), 8 (triangles), or 14 (squares) d after confluence. *a* represents 3T3-L1 fibroblasts and *b* 3T3-L1 cells subjected to induced adipose conversion as described in Materials and Methods.

bound layer exhibited a component with a slightly lower apparent relative molecular mass than the large proteoglycan found in the medium. Besides, the pooled fractions 24–35, especially from the fibroblasts, were dominated by material streaking between apparent relative molecular masses of 40 and 100 kD. This material may represent free glycosaminoglycans (Carlstedt et al., 1981; Fig. 6 *b*).

To get a more detailed picture of the time dependence of the rate of accumulation of proteoglycans in the medium as shown in Fig. 2 *a*, the size distribution of proteoglycans was characterized as a function of time in culture. In one initial series of experiments control fibroblasts and converted cells were harvested on the first, eighth, and fourteenth day after the day of the beginning of the conversion. After DE 52 chromatography of the media samples, the proteoglycan containing samples were subjected to chromatography on Superose 6. The most noticeable difference between the chromatograms of the samples of the two cell types was that the net rate of secretion of small proteoglycans in fraction 24–35 increased steadily in the fibroblasts and was 90% higher on the fourteenth day than on the first day (Fig. 7 *a*) while in the adipocytes the time dependence was negligible (Fig. 7 *b*).

Discussion

The differentiation of 3T3-L1 fibroblasts into adipocytes involves profound changes of several properties of the cells. Most obvious is the change of the morphology of the cells due to the accumulation of triglycerides intracellularly. This

accumulation is associated with a change of responsiveness to external stimuli as well as of the activity of several of the enzymes involved in lipid metabolism (Grimaldi et al., 1978; Fig. 1). The marked decrease of the accumulation of proteoglycans (Fig. 2) and hyaluronan (Fig. 3) is another event associated with the differentiation of these fibroblasts into adipocytes.

The synthesis of extracellular matrix components has previously been investigated in some other systems of differentiating cells. One cell type that has biological functions in part resembling those of the adipocytes is the fat storing cells in the liver. Such cells synthesize proteoglycans, of which those found in the medium are predominantly dermatan sulphate and chondroitin sulphate proteoglycans (Schäffer et al., 1987). In injured liver, the fat storing cells of the liver proliferate and transform into fibroblasts or transitional cells (Schäffer et al., 1987). The effect of this transformation on the synthesis of proteoglycans has not been reported.

By morphological criteria, 70–80% of the 3T3-L1 cells appeared as adipocytes at the time of harvest in the dishes where differentiation had been induced. Whether the remaining cells were fibroblasts or differentiated cells which simply have not accumulated fat is unclear. The critical question is whether the proteoglycans and the hyaluronan found in the adipocyte culture dishes originate from the adipocytes or not. The rate of accumulation of proteoglycans and hyaluronan increased with time in the control fibroblast cultures. If the adipocyte cultures had contained 20–30% fibroblasts one would expect to see an increase with time of the rate of proteoglycan and hyaluronan accumulation in the adipocyte cultures corresponding to that amount of fibroblasts. That was clearly not the case. Instead, the rate of accumulation of proteoglycans and hyaluronan in the adipocyte cultures decreased or was constant with time, respectively. Furthermore, the size distribution of secreted proteoglycans at various times in culture is clearly different between the two cell types and the pattern of proteoglycan synthesis is changed to give relatively smaller proportions of the small chondroitin/dermatan sulphate proteoglycan.

The change of proteoglycan metabolism upon the differentiation of the fibroblasts into adipocytes was most obvious for the chondroitin/dermatan sulphate proteoglycan species with an apparent M_r of 140 kD found in the medium (Fig. 6, lanes *a* and *b*). This size agrees with the size of proteoglycans of the PG-S2 type which have been reported to have a regulatory role for the formation of collagen fibers (Vogel et al., 1984; Hedbom and Heinegård, 1989) and a postulated role as a regulator for cell proliferation by altering the deposition of extracellular matrix components and interacting with transforming growth factor β (Yamaguchi et al., 1990). In contrast to the adipocytes, the rate of accumulation of this component in the fibroblast media after confluence increased steadily with time, thus marking the characteristics of a cell type the function of which is to organize and maintain the connective tissue matrix.

The proteoglycans in the 3T3-L1 cell cultures were predominantly of the chondroitin/dermatan sulphate type. Most of the ³⁵S label in the heparan sulphate proteoglycans was found in the cell layer. This agrees with the findings in other cell systems (Carlstedt et al., 1981). Pericellular heparan sulphate proteoglycans have important functions in interacting with fibronectin and contacting with the cytoskeleton of

the cells (Heremans et al., 1990). The accumulation of cell layer heparan sulphate proteoglycans was 60% lower in the adipocyte cultures and the reduction of the accumulation of PG-S2, which has a proposed inhibitory role in cell adhesion due to its interaction with fibronectin (Schmidt et al., 1987), was of the same magnitude. This could explain why we find it difficult to maintain 3T3-L1 fibroblasts but not adipocytes confluent in monolayer culture for >2 wk without the cells detaching, and it could also be of importance for the difference in shape between fibroblasts and adipocytes in addition to the decreased expression of tubulin and actin (Spiegelmann and Farmer, 1982), which is of importance for the organization of the cytoskeleton and its contact with the extracellular matrix (Woods et al., 1984).

Hyaluronan has a possible effect on cell adhesion (Turley, 1989) and binding proteins for hyaluronan in fibroblast cultures have been described (Tool et al., 1989; Underhill, 1989). The net rate of synthesis of hyaluronan in the medium and cell layer of adipocyte cultures was found to be 80% lower than in the corresponding control fibroblast cultures. This may explain the difference in cell adhesion between fibroblasts and adipocytes in culture.

In conclusion, it would seem reasonable for the adipocytes, once they lose their proliferative potential, only to produce enough extracellular matrix components to maintain good cell-substrate and cell-cell adhesion. With that view, the reduction of the net synthesis of extracellular matrix components after the conversion of fibroblasts into adipocytes would be a consequence of the conversion and the altered function of the cells. Yet another question remains to be clarified, and that is whether the reduction of the net synthesis of extracellular matrix components is a requirement for differentiation.

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References

- Aratani, Y., and Y. Kitagawa. 1988. Enhanced synthesis and secretion of type IV collagen and entactin during adipose conversion of 3T3-L1 cells and production of unorthodox laminin complex. *J. Biol. Chem.* 263:16163-16169.
- Bernlohr, D. A., C. W. Angus, M. D. Lane, M. A. Bolanowski, and T. J. Kelly. 1984. Expression of specific mRNAs during adipose differentiation: Identification of an mRNA encoding a homologue of myelin P2 protein. *Proc. Natl. Acad. Sci. USA.* 81:5468-5472.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* 72:248-254.

- Carlstedt, I., L. Cöster, and A. Malmström. 1981. Isolation and characterization of dermatan sulphate and heparan sulphate proteoglycans from fibroblast culture. *Biochem. J.* 197:217-225.
- Cöster, L., and L.-Å. Fransson. 1981. Isolation and characterization of dermatan sulphate proteoglycans from bovine sclera. *Biochem. J.* 193:143-153.
- Fischer, L. W., J. D. Termine, and M. F. Young. 1989. Deduced protein sequence of bone small proteoglycan I (biglycan) shows homology with proteoglycan II (decorin) and several nonconnective tissue proteins in a variety of species. *J. Biol. Chem.* 264:4571-4576.
- Fredrikson, G., P. Strålfors, N. Ö. Nilsson, and P. Belfrage. 1981. Hormone-sensitive lipase of rat adipose tissue. Purification and some properties. *J. Biol. Chem.* 256:6311-6320.
- Green, H., and O. Kehinde. 1974. Sublines of mouse 3T3 cells that accumulate lipid. *Cell.* 1:113-116.
- Green, H., and O. Kehinde. 1976. Spontaneous heritable changes leading to increased adipose conversion in 3T3 cells. *Cell.* 7:105-113.
- Green, H., and M. Meuth. 1974. An established pre-adipose cell line and its differentiation in culture. *Cell.* 3:127-133.
- Grimaldi, P., R. Negrel, and G. Ailhaud. 1978. Induction of the triglyceride pathway enzymes and of lipolytic enzyme during differentiation in a preadipocyte cell line. *Eur. J. Biochem.* 84:369-376.
- Hedbom, E., and D. Heinegård. 1989. Interaction of a 59-kDa connective tissue matrix protein with collagen I and collagen II. *J. Biol. Chem.* 264:6898-6905.
- Heremans, A., B. De Cock, J.-J. Cassiman, H. Van den Berghe, and G. David. 1990. The core protein of the matrix-associated heparan sulfate proteoglycan binds to fibronectin. *J. Biol. Chem.* 265:8716-8724.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* 227:680-685.
- Pairault, J., and H. Green. 1979. A study of the adipose conversion of suspended 3T3 cells by using glycerol-phosphate dehydrogenase as differentiation marker. *Proc. Natl. Acad. Sci. USA.* 76:5138-5142.
- Ronnett, G. V., V. P. Knutson, and M. D. Lane. 1982. Insulin-induced down-regulation of insulin receptors in 3T3-L1 adipocytes. *J. Biol. Chem.* 257:4285-4291.
- Rubin, C. S., A. Hirsch, C. Fung, and O. M. Rosen. 1987. Development of hormone receptors and hormonal responsiveness in vitro. *J. Biol. Chem.* 262:7570-7578.
- Schmidt, G., H. Robenek, B. Harrach, J. Glössl, V. Nolte, H. Hörmann, H. Richter, and H. Kresse. 1987. Interaction of small dermatan sulfate proteoglycan from fibroblasts with fibronectin. *J. Cell Biol.* 104:1683-1691.
- Schmidtchen, A., I. Carlstedt, A. Malmström, and L.-Å. Fransson. 1990. Inventory of human skin fibroblast proteoglycans. *Biochem. J.* 265:289-300.
- Spiegelman, B. C., and S. R. Farmer. 1982. Decrease in tubulin and actin gene expression prior to morphological differentiation of 3T3 adipocytes. *Cell.* 29:53-60.
- Schäfer, S., O. Zerbe, and A. M. Gressner. 1987. The synthesis of proteoglycans in fat storing cells of rat liver. *Hepatology.* 7:680-687.
- Toole, B. P., S. I. Munaim, S. Welles, and C. B. Knudson. 1989. Hyaluronate-cell interactions and growth factor regulation of hyaluronate synthesis during limb development. In *The Biology of Hyaluronan*. D. Evered and J. Whelan, editors. John Wiley & Sons Ltd., Chichester. 138-149.
- Turley, E. A. 1989. The role of a cell-associated hyaluronan-binding protein in fibroblast behaviour. In *The Biology of Hyaluronan*. D. Evered and J. Whelan, editors. John Wiley & Sons Ltd., Chichester. 187-106.
- Vogel, K. G., M. Paulsson, and D. Heinegård. 1984. Specific inhibition of type I and type II collagen fibrillogenesis by the small proteoglycan of tendon. *Biochem. J.* 223:587-597.
- Underhill, C. B. 1989. The interaction of hyaluronate with the cell surface: the hyaluronate receptor and the core protein. In *The Biology of Hyaluronan*. D. Evered and J. Whelan, editors. John Wiley & Sons Ltd., Chichester. 87-106.
- Woods, A., M. Höök, L. Kjellén, C. G. Smith, and D. A. Rees. 1984. Relationship of heparan sulfate proteoglycans to the cytoskeleton and extracellular matrix of cultured fibroblasts. *J. Cell Biol.* 99:1743-1753.
- Yamaguchi, Y., D. M. Mann, and E. Ruoslahti. 1990. Negative regulation of transforming growth factor- β by the proteoglycan decorin. *Nature (Lond.)* 346:281-284.