Steady-state Levels of mRNAs Coding for the Type IV Collagen and Laminin Polypeptide Chains of Basement Membranes Exhibit Marked Tissue-specific Stoichiometric Variations in the Rat*

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Rat retina, lens, and kidney from 8-week-old animals were assayed for the steady-state levels of mRNAs for four basement membrane components: The α1 chain of type IV collagen, the α2 chain of type IV collagen, the B1 chain of laminin, and the B2 chain of laminin. Each tissue exhibited markedly different ratios of the four mRNAs. The mRNA ratio for the α1 chain of type IV collagen to the B1 chain of laminin varied from a value of 0.7 in retina to a value of 17 in lens. Also, the mRNA ratio for the α1 chain to the α2 chain of type IV collagen varied from 1.6 in retina to 17 in lens, and the mRNA ratio for the B1 chain to the B2 chain of laminin varied from 0.6 in lens to 2.9 in kidney. The mRNA coding for the α1 chain of type IV collagen decreased in all three tissues as the animals increased in age from 8 to 16 weeks, with the rate of decrease being greater in retina than in lens or kidney. The levels of mRNA coding for the B1 and the B2 chains of laminin decreased in the kidney between 8 and 16 weeks but at different rates. Comparison of mRNAs from kidney of rats over this time period showed that the ratio of α1 to B1 remained relatively constant with age, whereas the ratio of B1 to B2 increased. One possible explanation for the results is that each tissue has elaborate, tissue-specific controls for translation that provide synthesis of basement membrane components in the same proportion, in spite of the varying steady-state levels of the mRNAs. A more likely explanation is that different tissues synthesize type IV collagen and laminin at different rates, and that even the subunit compositions of the type IV collagen and laminin molecules vary from tissue to tissue and in an age-dependent manner.

The investigation of BM metabolism in vivo has to date been based largely on studies examining rates of radiolabel incorporation and turnover (1–3), assays of enzymes involved in collagen post-translational modification (4–6), or measurement of serum levels of BM-derived antigens (7). These somewhat limited approaches were used because of the difficulty of isolation and inherent insolubility of BMs, and because of the very low levels of BM synthesis in most tissues.

The identification and characterization of cDNAs coding for polypeptide components of BM, such as the α1(IV) and α2(IV) chains of type IV collagen (8–12) and the laminin B1 and B2 chains (13), has provided a highly sensitive and specific means by which BM synthesis can be examined concurrently in different tissues, namely, by assaying the steady-state tissue concentrations of the mRNAs coding for these matrix proteins.

During the course of investigations to determine the effects of diabetes on BM synthesis in the rat (14), it became apparent that the retina, lens, and kidney exhibited marked but specific stoichiometric variations in the relative steady-state levels of these BM-coding mRNAs. This investigation addresses the quantitation of these tissue-specific variations and the changes in these mRNA levels that occur with age.

MATERIALS AND METHODS

RNA Isolation—Total RNA was isolated from the retina, lens, and kidney of male CDF rats (Charles River Laboratories, Wilmington, MA) by the guanidinium thiocyanate/cesium chloride method (15). RNA was shown to be intact by the presence of discrete 28 S and 18 S ribosomal bands after electrophoresis in formaldehyde (2.2 M) containing 15% agarose gels stained with ethidium bromide (15).

cDNA Probes and Labeling—The following mouse cDNAs were utilized in this investigation: pPE123 containing a 1.8-kb insert coding for the 204 amino acids at the C terminus of the NCI domain of the α1(IV) collagen chain and 1.2 kb of 3'-untranslated region (11), pPE18 containing a 1.1-kb insert coding for approximately 370 amino acids located within the central triple helix-forming region of the α2(IV) collagen chain (12), pPE36 containing a 1.1-kb insert coding for approximately 300 amino acids at the C terminus of the laminin B1 chain and 116 bases of 3'-untranslated region (13), and pPES containing a 0.7-bp base insert coding for the C-terminal end of the laminin B2 chain and 20 bases of 3'-untranslated region (13).

The inserts were removed from the described plasmids by restriction endonuclease digestion and routinely labeled with [32P]dCTP by nick translation to specific activities of 4–9 × 10^6 cpm/μg of DNA.

Northern and Slot-blotting Procedures—Northern analysis was performed by electrophoresis of heat-denatured RNA in a formaldehyde (2.2 M)-containing 15% agarose gel followed by capillary blotting onto nitrocellulose (15). For slot-blotting, known quantities of total RNA, assessed by absorbance at 260 nm, were denatured by heating in RNase-free H2O at 68°C for 15 min. The solutions were then rapidly chilled on ice, serially diluted, and applied to nitrocellulose filters in a denaturing buffer (6 × SSC, 2.5 M formaldehyde (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 6.8)) utilizing a slot-blotting apparatus (Minifold II, Schleicher & Schuell).

Hybridization Conditions—Northern and slot-blotted filters were air-dried, baked under vacuum at 80°C for 2 h, and prehybridized in 50% formamide, 4 × SSC, 1 mg/ml sheared salmon sperm DNA, 1 × Denhardt’s solution (14), and 0.1% SDS for 6 h at 37°C. Hybridiza-
ions with heat-denatured 32P-labeled cDNAs were conducted at 37°C for 24 h in the same solution as described for prehybridizations. Filters were washed to a final stringency of 0.2 × SSC and 0.1% SDS at 52°C.

 Autoradiograms were generated by exposure of the filters to x-ray film (Kodak XAR) in the presence of intensifying screens at −80°C. The bound label was assayed by absorbance per microgram of RNA from densitometric analysis of the autoradiographs.

 Statistics—Statistical analyses were performed by the two-tailed Student’s t test.

RESULTS

The Northern analysis of rat parietal endoderm total RNA (Fig. 1) demonstrates that, under the described hybridization and washing conditions, the mouse-derived cDNA inserts hybridize solely and specifically with their rat mRNA counterparts.

 Autoradiograms obtained from four identical slot-blots of total RNA obtained from the tissues of 8-week-old rats were hybridized with cDNAs coding for α1(IV) and α2(IV) chains of type IV collagen, and the B1 and B2 chains of laminin (Fig. 2). Changes in absorbance per microgram of RNA obtained from densitometric analysis of the autoradiographs together with stoichiometric comparisons are presented in Table I. It should be noted that, since the cDNAs utilized varied in length (0.6–1.8 kb) and specific activity (4–9 × 10⁶ cpm/µg of DNA), the ratios of mRNA levels presented are on an arbitrary rather than a molar scale.

 The lens and kidney exhibited steady-state levels of mRNA coding for α1(IV) chain that were on the order of 4 times higher per microgram of total RNA than that found in the retina (Fig. 2 and Table I). However, the steady-state levels of α2(IV) mRNA in lens and kidney were about half those of retina. Therefore, there was a markedly lower ratio of α1:α2(IV) chain mRNA in retina (Table I). All results presented were checked by reprobing the four slot-blots with the cDNAs in a different order after removal of the original label by treatment with 50% formamide and 0.1 × SSC at 65°C for 4 h.

 The kidney contained twice as much mRNA coding for B1 laminin per microgram of total RNA as did the retina, and more than 10 times as much as the lens (Fig. 2 and Table I). Laminin B2 mRNA analysis indicated that the kidney again

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Northern analysis of rat parietal endoderm total RNA hybridized with 32P-labeled mouse-derived BM-coding cDNAs. Total RNA (5 µg) from rat parietal endoderm was electrophoresed in a formaldehyde-containing agarose gel, blotted, hybridized, washed, and exposed to x-ray film under the same conditions subsequently used for slot-blotting (see "Materials and Methods"). The same filter was successively probed in the following order: 1) PE386 (laminin B1), 2) PE18 (α2(IV) collagen), 3) PE123 (α1(IV) collagen), and 4) PE9 (laminin B2). In each case, the previous 32P-labeled cDNA was removed by incubating the filter in 0.1 × SSC containing 50% formamide at 65°C for 4 h. The positions of the 28S and 18S ribosomal RNA bands are indicated as size markers.

![Table I](https://example.com/table1.png)

**Table I**

| Type IV collagen mRNA | Laminin B1 mRNA | Ratio of mRNAs |
|----------------------|-----------------|----------------|
| α1 α2 | α1α2 | B1 B2 | α1α2 | B1:B2 | α1:B1 |
| Retina | 0.13 | 0.08 | 0.18 | 0.10 | 2.9 | 1.2 |
| Lens | 0.51 | 0.03 | 0.03 | 0.05 | 0.6 | 0.7 |
| Kidney | 0.48 | 0.04 | 0.40 | 0.14 | 17 | 17 |

To illustrate the variations from tissue to tissue, the results were calculated from a single autoradiograph prepared with equal amounts of RNA from the three tissues (see Fig. 2). Values for mRNA are calculated from densitometric analysis of the autoradiograph and are expressed in arbitrary absorbance units per µg of RNA. As indicated in Tables II and III, the S.E. for the assays is about ±30% of the mean. As indicated in the text, approximately the same values for the ratio of mRNAs were obtained when the same filters were reprobed in a different order with a new set of nick-translated cDNA probes.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Autoradiographs of slot-blotted total RNAs hybridized to 32P-labeled cDNAs for type IV collagen and laminin. Total RNA extracted from retina, lens, and kidney was slot-blotted on nitrocellulose filters in the amounts indicated. The four identical blots were hybridized with nick-translated cDNAs for the two chains of type IV collagen (PE123α1 chain, PE18α2 chain) and two of the laminin chains (PE386B1, PE9B2). After washing, the filters were exposed to x-ray film for 24 h as described under "Materials and Methods."

**Table II**

Relative steady-state levels of mRNAs for α1 and α2 chains of type IV collagen and the B1 and B2 chains of laminin at 8 weeks of age

To determine how these levels change as the rat increases in age over the period of 8–16 weeks, Table II presents the changes in the steady-state levels of α1(IV) collagen mRNA levels in each tissue examined and varied over a 5-fold range (Table I).

The relative levels of type IV collagen to laminin mRNAs in the lens were far greater than that exhibited by either the retina or the kidney (Table I). On the basis of the data presented in Table I, the retina appears to synthesize BM with the lowest levels of type IV collagen relative to laminin B chains.

Having established that each of the three tissues examined in 8-week-old rats exhibited its own specific pattern of steady-state levels of BM-coding mRNAs, we proceeded to investigate how these levels change as the rat increases in age over the period of 8–16 weeks. Table II presents the changes in the steady-state levels of α1(IV) collagen mRNA levels in each tissue over this time period. The steady-state levels of α1(IV) collagen decreased in all three tissues between 8 and 16 weeks of age. However, in the lens and kidney, the α1(IV) collagen mRNA level decreased to about half between 8 and 16 weeks,
TABLE II

| Age (weeks) | mRNA Levels |
|-------------|-------------|
| Retina      | Lens        | Kidney     |
| 8           | 138 ± 4     | 100 ± 37e | 100 ± 4e  |
| 10          | 192 ± 1     | 42 ± 16   | 87 ± 15   |
| 12          | 236 ± 8     | 48 ± 7    | 84 ± 21   |
| 16          | 294 ± 4     | 11 ± 3d   | 54 ± 15f  |

The B1:B2 laminin ratio exhibited a marked increase (Fig. 3).

TABLE III

| Age (weeks) | mRNA Levels |
|-------------|-------------|
| B1          | B2          |
| 8           | 100 ± 23e   | 100 ± 21e |
| 10          | 122 ± 12    | 79 ± 10   |
| 12          | 85 ± 12d    | 31 ± 3e   |
| 16          | 72 ± 16e    | 26 ± 3e   |

The results obtained from 8-week-old rats establish that the ratio of the steady-state levels of mRNAs for type IV collagen to laminin vary over a 20-fold range among retina, lens, and kidney. In addition, they demonstrate that the ratio of the steady-state levels of mRNAs for the two polypeptide chains of type IV collagen and for two of the chains of laminin vary over a 5-10-fold range in the same tissues. These differences were tissue-specific and also dependent on age. The a1 type IV collagen mRNA level in retina decreased with age at a greater rate than that noted in lens or kidney. Furthermore, kidney B1 and B2 laminin mRNA levels also decreased with age but at markedly different rates. This resulted in a steady increase of the ratio of B1 to B2 laminin mRNA levels in this tissue with age, whereas the a1(IV) collagen to B1 laminin mRNA ratio exhibited no consistent trend.

Steady-state levels of mRNA do not always reflect rates of protein synthesis. Therefore, it is possible that the large variations seen here in the ratios of mRNAs are compensated for by differences in the rates of translation so as to generate the components in the same stoichiometry in all tissues. This, however, would require an elaborate series of controls for translation of specific mRNAs. Therefore, the simplest explanation for the data presented herein is that the tissues synthesize the matrix components at different rates and that the BMs contain different amounts of type IV collagen and laminin. Also, the data are consistent with the conclusion that the chain composition of both the type IV collagen molecule and laminin molecule varies from tissue to tissue. Furthermore, the kidney data suggest that the chain composition of laminin may vary within a particular tissue as a function of age. Thus, tissues appear to regulate their BM-coding mRNA levels differently.
levels in such a way as to allow the synthesis and composition of their BMs to adapt to meet their own physiological requirements at any specific point in time.

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