Coordinate Gene Expression of the α3, α4, and α5 Chains of Collagen Type IV

EVIDENCE FROM A CANINE MODEL OF X-LINKED NEPHRITIS WITH A COL4A5 GENE MUTATION*

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Canine X-linked hereditary nephritis is an animal model for human X-linked hereditary nephritis with a premature stop codon in the α5(IV) gene of collagen type IV. We used this model to examine the other α(IV) chains at the mRNA and protein level in the kidney, since in human X-linked hereditary nephritis, the α3(IV) and α4(IV) chains are often absent from the glomerular basement membrane, although both are encoded by autosomal genes. cDNA probes for the α1(IV)–α6(IV) chains were generated from normal dog kidney using the polymerase chain reaction. Sequences were 88% identical at the DNA level and 92% identical at the protein level to the respective human α(IV) chains. By Northern analysis, transcripts for the α1(IV), α2(IV), and α6(IV) chains were detected at comparable levels in both normal and affected male dog kidney RNA. As previously shown, the transcript for the α5(IV) chain was reduced to ~10% of normal. Unexpectedly, the α3(IV) and α4(IV) transcripts were both decreased 77% in affected male dog kidney, suggesting a mechanism coordinating the expression of these three basement membrane components. The NC1 domain of collagen type IV isolated from normal dog glomeruli was positive for the α3(IV), α4(IV), and α5(IV) chains by Western blotting. In contrast, in the NC1 domain isolated from affected dog glomeruli, these three chains were not detectable, except for a trace of α3(IV) dimer. In X-linked hereditary nephritis, the absence of the α3(IV) and α4(IV) chains from glomerular basement membrane may reflect factors acting at the transcriptional and/or translational level in addition to the protein assembly level.

Hereditary nephritis (HN)† refers to a group of genetic disorders of collagen type IV with glomerular disease that often progresses to renal failure and sometimes extrarenal disease such as high tone sensorineural hearing loss and anterior lenticonus (1, 2). About 80% of affected families show X-linked inheritance; the remainder are autosomal dominant or recessive (3–5). Patients present from childhood to early adulthood, usually with hematuria. Most male patients develop terminal renal failure by the end of the third decade, while female patients range from no renal dysfunction to terminal renal failure. The most characteristic morphologic finding in HN is multilaminar splitting of glomerular basement membranes (GBM) as seen by electron microscopy (6). Type IV collagen, the major component of basement membranes, has been linked to the pathogenesis of both X-linked and autosomal HN.

Type IV collagen has recently emerged as a family of triple helical isoforms consisting of six genetically distinct chains, designated α1(IV)–α6(IV) (7). The entire coding sequences for all six human chains have now been determined (8–14). Their primary structures are similar; each is characterized by a ~25-residue noncollagenous sequence at the amino terminus, a long collagenous domain of ~1400 residues of Gly-X-Y repeats that forms the triple helix together with two other α chains, and a ~230-residue noncollagenous (NC1) domain at the carboxy terminus. The existence of six α chains allows for 56 different kinds (isoforms) of triple helical molecules, which differ in type and stoichiometry of chains. Evidence has been obtained for heterotrimers that have chain compositions of (α1)α2 and (α3)α44 and homotrimers of (α1)3 and (α3)3 (7). Isoforms containing the α5 or α6 chains have not yet been described.

The genes that code for the six human α(IV) chains have a unique arrangement in that they are located pairwise in a head-to-head fashion on chromosome 13 (COL4A1 and COL4A2) (15), chromosome 2 (COL4A3 and COL4A4) (16), and the X chromosome (COL4A5 and COL4A6) (17, 18). Over 50 mutations have been found in the COL4A5 gene in families with X-linked HN (Alport syndrome (reviewed in Ref. 19), and mutations have been found in the COL4A3 and COL4A4 genes in patients with autosomal recessive HN (5, 20). Although the specific effects of each mutation are not well understood at the molecular level, the end result of all of them is predicted to be an abnormal collagen type IV molecule. This presumably leads to formation of abnormal GBM and progressive renal disease.

How COL4A3, COL4A4, and COL4A5 gene mutations alter the structure of type IV collagen remains undefined. In normal GBM, this collagen is composed of five α(IV) chains (α1–α5) with α1 and α2 chains forming one kind of triple helical isoform, α3 and α4 chains another kind, and α5 chains an undefined kind. In the abnormal Alport GBM, there are several lines of evidence suggesting that COL4A5 gene mutations cause defective assembly of the α3 chain (reviewed in Ref. 21), and in one study defective assembly of the α4 chain was re-
ported (22). This conundrum leads to the hypothesis that a mechanism exists for the synthesis of normal GBM that links the assembly, either at the mRNA or protein level, of triple helical molecules containing the α3(IV) chain with triplehelical molecules containing the α5(IV) chain.

The purpose of the present study was to determine the influence of COL4A5 mutation on the expression of α1(IV) chains at the mRNA and protein levels. This was accomplished using a unique family of Samoyed dogs with an X-linked form of HN, which closely resembles human X-linked HN at the clinical, genetic, morphologic, and immunohistochemical levels (23–27) and which is caused by a single base substitution in the COL4A5 gene that results in a premature stop codon (28). The findings indicate the existence of a mechanism coordinating the expression of the α3(IV), α4(IV), and α5(IV) chains.

MATERIALS AND METHODS

Preparation of RNA from Dog Kidney—Normal and affected dogs (paired littermates born to two different carrier females) were sacrificed at 4 months of age, and samples of kidney tissue were snap-frozen in liquid nitrogen. Total RNA was prepared from 1 g of kidney tissue by lysis in guanidinium isothiocyanate followed by centrifugation over 5.7 M CsCl, as described previously (28).

cDNA Synthesis and Amplification by the Polymerase Chain Reaction (PCR)—Synthesis of first strand cDNA was carried out using normal dog RNA according to Sambrook (29). The canine α2(IV), α3(IV), and α6(IV) cDNAs for the NC1 domains were obtained using nested PCR reactions. Pairs of synthetic oligonucleotide primers for the respective cDNAs were constructed based on the sequence of the human α2(IV) cDNA (9), the sequence of the bovine α3(IV) cDNA (30), and the sequence of the human α6(IV) cDNA (14).

The sequences for the α2(IV) primers used were as follows.

**External**

- **Sense** 5'-TTGGCCGAAAGGGCCACCA-3'
- **Antisense** 5'-CAGGGTTCTCCATCGACC-3'

**Internal**

- **Sense** 5'-ATCAGCTCTTCCTCGTGA-3'
- **Antisense** 5'-GCTGATGTGGTGCCGGA-3'

The sequences for the α3(IV) primers used were as follows.

**External**

- **Sense** 5'-GCAGCAGGGGCGATGAGG-3'
- **Antisense** 5'-GTCTTCATCCTCATGCAC-3'

**Internal**

- **Sense** 5'-GATGAGGGGGCTTGTGT-3'
- **Antisense** 5'-TGACACTTGCAGGAC-3'

The sequences for the α6(IV) primers used were as follows.

**External**

- **Sense** 5'-TCCGGATGCTGGGATACCTG-3'
- **Antisense** 5'-GCTTTCATCATAACACCTGC-3'

**Internal**

- **Sense** 5'-CATGAGAGTGGGCGATGAG-3'
- **Antisense** 5'-AGCTGACAGGTGTCAGGAC-3'

The first round of PCR was performed using 1 µl of the 20 µl of first strand cDNA prepared above as template and 100 ng of each of the primers in Perkin-Elmer PCR buffer containing 2.5 mM MgCl2 to a total volume of 100 µl. Samples were denatured at 95 °C for 3 min and cooled to 80 °C and 0.5 µl of Taq polymerase (2.5 units) added. 35 cycles were carried out in a Perkin-Elmer DNA thermal cycler. Each cycle consisted of denaturation at 94 °C for 1 min, annealing at 55 °C, and extension at 72 °C for 2 min.

Southern Blot Analysis—Genomic DNA from Chinese hamster and a human-Chinese hamster somatic cell hybrid with single human 2, 13, or X as the only human chromosomes were purchased from Coriell (Camden, N.J.). Genomic DNA samples from a normal male and female, After digestion of each sample were cleaved with EcoRI for the α1(IV) and α3(IV) genes, PstI for the α2(IV), α4(IV), and α6(IV) genes. Southern analysis was performed as described previously (28) using the NC1 domain cDNAs obtained above by PCR as probes for the α1(IV)–α4(IV) and α6(IV) genes. The probes were labeled with [32P]dCTP by random primer synthesis (32).

Nucleotide Sequencing—The amplified PCR products were purified by Magic PCR Prep DNA Purification System (Promega, Madison, WI) according to the manufacturer’s instructions. The cDNAs encoding the NC1 domains of the various α(IV) chains were obtained by subcloning the PCR products into the TA cloning vector (Invitrogen, San Diego, CA) and then using M13 universal primer and “Sequenase” (U.S. Biochemical Corp.), according to the manufacturer’s instructions.

Northern Blot Analysis and Densitometry—Ten-µg samples of total RNA prepared from normal and affected male dogs were separated by electrophoresis and blotted as described previously (28). The probes were the PCR products encoding the NC1 domain of the α1(IV)–α4(IV) and α6(IV) cDNAs labeled with [32P]dCTP as described above, as well as the PCR product encoding the NC1 domain of the α5(IV) cDNA (28) and actin. The gel was run in duplicate. Hybridization signals from the autoradiographs were quantitated using a Molecular Dynamics computer system. The signals from the α(IV) transcripts were quantitated relative to the signal from the actin transcript in order to control for variations in gel loading. All measurements were taken at two different exposure times.

Preparation of NC1 Domain from Dog Kidney—Glyceraldehyde from the kidneys of normal and affected dogs at 4 months of age were isolated by graded sieving as described previously (33). Equal amounts of normal and affected globulin (1 mg) were digested with bacterial collagenase for 24 h at 37 °C (34). The collagenase-solubilized fraction was recovered after centrifugation and collection of the supernatant. The protein in the supernatant was quantitated using the spectrophotometric absorbance at 280 nm.

Western Blotting—One-dimensional SDS-PAGE analysis was performed to confirm equal concentrations of NC1 domain in the normal and affected collagenase-solubilized GBM. Western blotting was performed as described previously (34). The human antibodies used included serum from a patient with Goodpasture syndrome (diluted 1:100) and serum from a patient with HN who received a renal transplant and subsequently developed anti-GBM antibodies (diluted 1:1000). Both of these sera have been previously characterized to be directed against the α3(IV) chain of collagen type IV (35). The rab antibodies used were directed against the α3(IV), α4(IV), and α5(IV) chains of collagen type IV as described previously (36) (diluted 1:200).

Immunofluorescence—Immunofluorescence was performed as previously reported (26) using the two human sera described above. Sections were pretreated with 6 µM urea, pH 3.5. The serum from a patient with Goodpasture syndrome was used at a dilution of 1:10 and the serum from the HN patient was used at a dilution of 1:20.
RESULTS

Experimental Approach—In order to determine the influence of a COL4A5 mutation on the expression of α(IV) chains at the mRNA and protein levels, we used the Samoyed dog model of HN in which there is a premature stop codon in the COL4A5 gene and almost no α5(IV) mRNA (28). In order to address the expression of the various α(IV) chains at the mRNA level, it was necessary to clone the cDNA for the NC1 domain for each chain. Each of these cDNAs was then characterized at two levels: 1) the degree of cross-hybridization to the other α(IV) genes using rodent-human hybrid preparations, and 2) the nucleotide and derived amino acid sequence. After establishing that each cDNA was derived from its respective α(IV) gene, these cDNAs were used as probes to determine the message levels for the α1(IV)–α6(IV) chains, comparing normal and affected dogs. Based on these results, experiments at the protein level were designed to compare the NC1 domain prepared from normal and affected dog glomeruli. Initially, two patient sera were used, one from a patient with Goodpasture syndrome, the other from a HN patient who had undergone a renal transplant, both known to be directed against the α3(IV) chain. These results were then refined by using antibodies specific for each of the α3(IV), α4(IV), and α5(IV) chains.

Chromosome Localization of the Amplified Canine cDNAs—To establish that each of the amplified cDNAs was encoded by its own α(IV) genes rather than some other collagen gene, we examined hybridization of these cDNAs to genomic DNA samples by Southern blot analysis. The cDNAs encoding the canine α1(IV) and α2(IV) NC1 domains hybridized to total genomic DNA from normal human male (Fig. 1, lane 1) and female (lane 2), and to total genomic DNA from Chinese hamster (lane 4). In the genomic DNA sample of the human-Chinese hamster somatic cell hybrid with chromosome 13 as the only human chromosome (lane 3), all the human and hamster bands were present (Fig. 1, a and b). In similar experiments, the cDNAs encoding the canine α3(IV) and α4(IV) NC1 domains hybridized to human chromosome 2 (Fig. 1, c and d) and the cDNA encoding the canine α6(IV) NC1 domain hybridized to human chromosome X (Fig. 1e). No cross-hybridization to other collagen genes was seen with any of the canine α(IV) cDNAs.

Comparison of Nucleotide and Deduced Amino Acid Sequences of the Normal Canine and Human α1(IV)–α4(IV) and α6(IV) NC1 Domains—Sequence analysis of the PCR fragment provided the nucleotide sequence for 676 bp of the canine α1(IV) cDNA, 608 bp of the α2(IV) cDNA, 633 bp of the canine α3(IV) cDNA, 627 bp of the α4(IV) cDNA, and 618 bp of the canine α6(IV) cDNA. These sequences have been deposited in GenBank™. The deduced amino acid sequences are presented in Fig. 1. The sequence for canine α1(IV) chain included 6 residues of the collagenous region and 219 residues of the NC1 domain, lacking 10 residues at the C terminus, assuming a length of 229 residues as in the human α1(IV) NC1 domain (8). The sequence for canine α2(IV) chain included 202 residues of the NC1 domain, lacking 9 residues at the N terminus and 16 residues at the C terminus, assuming a length of 227 residues as in the human α2(IV) NC1 domain (9). The sequence for canine α3(IV) chain included 210 residues of the NC1 domain, lacking 11 residues at the N terminus and 12 residues at the C terminus, assuming a length of 233 residues as in the bovine α3(IV) NC1 domain (30) or 10 residues at the N terminus, assuming a length of 232 residues as in the human α3(IV) NC1 domain (37). The sequence for canine α4(IV) chain consisted of 208 residues of the NC1 domain, lacking 11 residues at the N terminus and 12 residues at the C terminus, assuming a length of 231 residues as in the bovine and human α4(IV) NC1 domains (16, 38). The sequence for canine α6(IV) chain included 205 residues of the NC1 domain, lacking 7 residues at the N terminus and 16 residues at the C terminus, assuming a length of 228 residues as in the human α6(IV) NC1 domain (14).

The percentage of identity between corresponding canine and human sequences is shown in Table I. Of note, the positions of the cysteine residues are conserved between all canine α(IV) chains and between each canine and human α(IV) chain. The percentage of identity at the amino acid level between the canine α1(IV)–α6(IV) NC1 domains is shown in Table II.

Northern Analysis of Canine α1(IV)–α6(IV) mRNAs—The cDNAs for the NC1 domains for the α1(IV)–α6(IV) chains obtained above were used as probes to determine the level of the messages for their respective genes, comparing normal and affected dog kidney. For the α1(IV) mRNA, transcripts were present at 6.8, 6.1, and 5.5 kb in both normal and affected dog kidney, at comparable levels when measured by densitometry (Fig. 3a). For the α2(IV) mRNA, a single transcript was present...
at 6.4 kb in both normal and affected dog kidney, at similar levels when measured by densitometry (Fig. 3b). The mRNAs are comparable in size with the values of 6.7 and 5.4 kb reported for the human a1(IV)mRNA (39) and 6.4 kb reported for the a2(IV) mRNA (40).

Using the canine a3(IV) NC1 domain cDNA as a probe, a single transcript of ~8 kb was identified in both normal and affected male dog kidney RNA (Fig. 3c). This size is the same as that reported for human a3(IV) mRNA (37). By densitometry, the abundance of the a3(IV) transcript in the affected dog was 14–23% that seen in normal dog. When using the canine a4(IV) NC1 domain cDNA as a probe, a single transcript of ~10 kb was identified in both normal and affected male dog kidney RNA (Fig. 3d). This size is the same as that reported for human a4(IV) mRNA (38). The abundance of the a4(IV) transcript in the affected dog was 11–17% that seen in normal dog as determined by densitometry.

As reported previously (28), using the canine a5(IV) NC1 domain cDNA, a minor transcript at ~8.6 kb and a major transcript at ~6.7 kb were identified in both normal and affected male dog kidney RNA (Fig. 3e). As before, the abundance of both a5(IV) transcripts was decreased ~90% in the affected dog as determined by densitometry. The canine a6(IV) NC1

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**Table I**

**Comparison of human and canine NC1 domain sequences**

| % identity at: | a1(IV) | a2(IV) | a3(IV) | a4(IV) | a5(IV) | a6(IV) |
|---------------|--------|--------|--------|--------|--------|--------|
| cDNA level    | 88     | 91     | 90     | 88     | 90     |        |
| Amino acid level | 96     | 98     | 92     | 93     | 95     |        |
| Reference     | 8      | 9      | 37     | 38     | 14     |        |

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**Table II**

% identity at amino acid level between the canine NC1 domain

| % identity at amino acid level | a2(IV) | a3(IV) | a4(IV) | a5(IV) | a6(IV) |
|-------------------------------|--------|--------|--------|--------|--------|
| a1(IV)                        | 66     | 71     | 60     | 82     | 64     |
| a2(IV)                        | 62     | 71     | 64     | 75     |        |
| a3(IV)                        | 53     | 71     | 59     |        |        |
| a4(IV)                        | 57     | 67     |        |        |        |
| a5(IV)                        |        |        |        |        | 64     |
domain cDNA hybridized to transcripts of 7.3 and 6.6 kb in both normal and affected dog kidney (Fig. 3f). This value is comparable with the 6.8–7.3-kb values reported for the human α6(IV) mRNA (13, 14). By densitometry, the level of message in the affected dog was 74–80% of normal.

Western Blotting of Canine NC1 Domain—The serum from the patient with Goodpasture syndrome and the serum from the HN patient who developed anti-GBM antibodies after renal transplantation gave similar results. Both sera stained a single band in the dimer and monomer regions of normal dog NC1 domain, while no staining was seen in the case of affected male dog NC1 domain (Fig. 4a). The control sera did not exhibit any specific binding. When chain-specific antibodies for the α3(IV), α4(IV), and α5(IV) chains were used, each chain was detected in normal dog NC1 domain as single bands in both the dimer and monomer band regions. In the case of the NC1 domain prepared from affected dog kidney, only a very faint band in the dimer region could be detected for the α3(IV) chain. No bands for the α4(IV) and α5(IV) chains were seen (Fig. 4b).

Immunofluorescence Studies—The serum from the patient with Goodpasture syndrome and the serum from the HN patient who developed anti-GBM antibodies after renal transplantation gave similar results. Linear staining of capillary loops of normal dog glomeruli was seen, but there was no staining in glomeruli of affected male dogs (Fig. 5).

**DISCUSSION**

The sequences of the human α1(IV), α2(IV), and α6(IV) chains and the bovine α3(IV) and α4(IV) cDNAs were used to obtain the equivalent canine cDNAs by PCR. The canine PCR products were established to be encoded by their respective canine genes in two ways. First, all PCR products hybridized to the appropriate human chromosome with no cross-hybridization. Second, the sequences of each canine αIV cDNA showed over 88% identity to the bovine and/or human NC1 domains at both the nucleotide and amino acid levels. The positions of all cysteine residues were conserved both between species and between canine αIV chains. In general, the amino acid sequences were more similar among the α1(IV), α3(IV), and α5(IV) chains (71–82% identity) and among the α2(IV), α4(IV), and α6(IV) chains (67–75% identity) than with the other αIV chains (53–66% identity). These results are in keeping with the concept that the αIV chains fall into two families based on sequence similarities, with the α1(IV), α3(IV), and α5(IV) chains in one family and the α2(IV), α4(IV), and α6(IV) chains in another (18). The sequences for the normal canine α3(IV) and α4(IV) chains will provide a base line of comparison for the situation where single amino acid substitutions are found in human families with autosomal recessive HN (5, 20), in order to ascertain whether these changes are more likely pathogenic or simply polymorphisms.

In the X-linked form of HN, there are several observations that indicate that the α3(IV) and α4(IV) chains are abnormal even though both are encoded by autosomal genes (reviewed in Ref. 21). It is unclear how a mutation in the α5(IV) gene accounts for the abnormalities in these other αIV chains, but implied is the existence of one or more mechanisms that link the incorporation of the α3(IV), α4(IV), and α5(IV) chains, and that could operate at the protein assembly and/or at the translational/transcriptional level (35). At the protein level, events at both triple helix formation and supramolecular assembly need to be considered. Should the α3(IV), α4(IV), and α5(IV) chains form heterotrimers, then an abnormal α5(IV) chain could lead to faulty heterotrimer assembly, with resultant absence of these chains in GBM of patients with X-linked HN. Should the α3(IV) and α4(IV) chains be in trimers distinct from those containing the α5(IV) chain, the latter may be necessary for incorporation of α3(IV) and α4(IV)-containing trimers during the supramolecular assembly of collagen type IV molecules. An abnormality of α5(IV)-containing trimers in X-linked HN could then lead to absence of the α3(IV)- and α4(IV)-containing trimers from GBM. At the translational/transcriptional level,
syndrome gave identical results (not shown).

We found that the levels of mRNA for the COL2A1 gene have RNA helicase activity and affect ribosome and splicing. The a3(IV), a4(IV), and a5(IV) genes in the kidney are currently not understood and could include factors acting at the gene (transcriptional) level or at the mRNA (translational) level. The a3(IV) and a4(IV) genes are both on chromosome 2 (46–48); however, message levels for the a3(IV), a4(IV), and a5(IV) genes have not been determined, likely the consequence of limited material from patient biopsies, at the same time underscoring the advantage of an animal model. In other collagen types, premature stop codons have been identified in COL2A1 gene in Stickler syndrome (arthro-ophthalmapathy) (49), but message levels have not been reported. In dystrophic epidermolysis bullosa, a homozygous mutation resulting in a premature stop codon in both alleles of the COL7A1 gene has been reported (50). In this case, there was a marked reduction in the message levels for the COL7A1 gene and no detectable a1(VII) chain. Since both collagen type II and type VII are homotrimers, the message levels of other collagen chains on different chromosomes is not relevant. In osteogenesis imperfecta type I, a premature stop codon in the mutant allele results in only about half of the normal amount of collagen type I being made. There is a marked reduction in the amount of message from the mutant COL1A1 allele, with normal amounts of mRNA produced from the normal COL1A1 allele and the two normal COL1A2 alleles (51, 52).

The reductions we detected for the a3(IV), a4(IV), and a5(IV) chains at the message level were then investigated at the protein level, first by using spontaneously occurring antibodies in patient sera directed against the a3(IV) chain (35, 36) to confirm that a similar situation exists in canine X-linked HN as seen in human X-linked HN, namely affected male dogs show absence of the a3(IV) chain both by Western blotting and by immunofluorescence. These results correlate with our previous immunofluorescence results in which other sera from patients with Goodpasture syndrome (25) and anti-GBM nephritis (26) failed to stain GBM of affected male dogs. We next refined these studies by performing Western blotting with chain-specific antibodies. The a3(IV), a4(IV), and a5(IV) chains were present in GBM of normal dogs but undetectable in affected male dogs other than a faint dimer band for the a3(IV) chain. Thus, a comparable situation exists with canine and human X-linked HN, namely a primary mutation in the a5(IV) gene is associated with virtual absence of the a3(IV) and a4(IV) chains as well as the a5(IV) chain.

The mechanisms that might lead to decreased message levels for the a3(IV), a4(IV), and a5(IV) chains in a coordinate fashion are currently not understood and could include factors acting at the gene (transcriptional) level or at the mRNA (translational) level. The a3(IV) and a4(IV) genes are in a head-to-head arrangement and share a bidirectional promoter located between the two genes and an enhancer element in intron 1 of the a3(IV) gene (40, 53). Transcription factors and specific DNA motifs that increase transcriptional activity have been identified in both the promoter region (54–56) and the enhancer region (56–58). Recently, it has been shown that this common promoter region is not equally bidirectional and is more correctly viewed as two overlapping promoters that share common elements but are unidirectional and gene-specific (55, 56). The a3(IV) and a4(IV) genes are both on chromosome 2 (16), and it is reasonable these two genes share regulatory elements. Should similar motifs be present in the promoter regions of the a3(IV), a4(IV), and a5(IV) genes (currently unknown), there would be a potential molecular basis for coordinate transcriptional control. Still, it would be difficult to explain how, through shared positive transcription factors, a premature stop codon in one a1(IV) chain could then bring about decreased transcription of two other a1(IV) chains. Instead, one would need to hypothesize increased levels of some negative or inhibitory transcription factor. For instance, such a factor (protein) might bind to regulatory elements of the a3(IV) and a4(IV) genes and to the a5(IV) mRNA in an equilibrium state. When the a5(IV) mRNA is being produced, the protein would tend to bind to this message instead of the a3(IV) and a4(IV) genes, thereby derepressing the transcription of these two genes. When the a5(IV) mRNA is virtually absent, as in the case of canine X-linked HN, the protein stays bound to the a3(IV) and a4(IV) genes, repressing their transcription.

It has also been shown that DNA methylation of the 5'-flanking region of the a1(IV) gene is associated with increased transcription (59). The relevance of this finding to the other a(IV) genes remains to be determined. Recently, a group of proteins known as DEAD box proteins have been recognized that have RNA helicase activity and affect ribosome and spli-
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References

1. Habib, R. G., Rubler, M. C., Hinglais, N., Noël, L.-H., Droz, D., Levy, M., Mahieu, P., Fraitag, J., Mierrin, D., Bais, E., and Grünfeld, J. P. (1982) Kidney Int. 21, Suppl. 11, 520–528.

2. Grünfeld, J. P. (1985) Kidney Int. 27, 83–92.

3. Fraitag, J., Bois, E., Choppet, L., Broger, M., Rubler, M. C., and Grünfeld, J. P. (1985) Kidney Int. 27, 672–677.

4. Reenders, S. (1992) Kidney Int. 42, 783–792.

5. Lemmink, H. H., Mochizuki, T., van den Heuvel, L. P. W. J., Sjöder, C. H., Bariart, A., Monnier, V. M., and Verheijen, R. H., van Oost, B. A., Brunner, H. G., Reenders, S. T., and Smeets, H. J. M. (1994) Hum. Mol. Genet. 3, 1269–1273.

6. Spear, G. S., Slusser, R. J. (1972) Am. J. Pathol. 65, 213–224.

7. Hudson, B. G., Reenders, S. T., and Tryggvason, K. (1993) J. Biol. Chem. 268, 26303–26306.

8. Brael, D., Oberbauer, I., Dieringer, H., Babel, W., Glanville, R., Deutzmann, R., and Kuhn, K. (1987) Eur. J. Biochem. 169, 529–536.

9. Hostiuk, S. L., and Tryggvason, K. (1988) Biochem. 263, 19488–19493.

10. Zhou, J., Hertz, J. M., Leinonen, A., and Tryggvason, K. (1992) J. Biol. Chem. 267, 12475–12481.

11. Mochizuki, T., Leinonen, A., Mochizuki, T., Tryggvason, K., and Reenders, S. T. (1994) J. Biol. Chem. 269, 23013–23017.

12. Leinonen, A., Mochizuki, M., Mochizuki, T., Tryggvason, K., and Reenders, S. T. (1994) J. Biol. Chem. 269, 23161–23177.

13. Ohashi, T., Sugimoto, M., Mattel, M.-G., and Ninomiya, Y. (1994). Biochem. 269, 7520–7526.

14. Zhou, J., Ding, M., Zhao, Z., and Reenders, S. T. (1994) J. Biol. Chem. 269, 13193–13199.

15. Griffin, C. A., Emanuel, B. S., Hansen, J. R., Caveness, W. K., and Myers, J. C. (1987) Proc Natl Acad Sci USA 84, 512–516.

16. Mochizuki, T., Kalluri, R., Hudson, B. G., and Reenders, S. T. (1992) J. Biol. Chem. 267, 1253–1258.

17. Hostiuk, S. L., Eddy, R. L., Byers, M. G., Hoyhtya, M., Shows, T. B., and Tryggvason, K. (1990) Proc Natl Acad Sci USA 87, 1606–1610.

18. Zhou, J., Mochizuki, T., Smeets, H., Antignac, C., Laurila, P., de Paepe, A., Tryggvason, K., and Reenders, S. T. (1993) Science 261, 1167–1169.

19. Tryggvason, K., Zhou, J., Hostiuk, S. L., and Shows, T. B. (1993) Kidney Int. 43, 38–44.

20. Mochizuki, T., Lemmink, H. H., Mochizuki, M., Antignac, C., Rubler, M.-P., Visseren-Dumoulin, C., Chan, B., Sjöder, C. H., Smeets, H. J. M., and Reenders, S. T. (1994) Kidney Int. 45, 77–82.

21. Kalluri, R., Weber, M., Netzzer, K.-O., Sun, M. J., Neilson, E. G., and Hudson, B. G. (1994) Kidney Int. 45, 721–726.

22. Kleppe, M. M., Kashan, G., Butkovic, R. J., Fish, A. J., and Michael, A. F. (1987) J. Clin. Invest. 80, 263–266.

23. Jansen, B., Thorner, P., Baumal, R., Valli, V., Maix, M. G., and Singh, A. (1986) Am J Pathol. 121, 517–528.

24. Jansen, B., Tryphonas, L., Valli, V. E., Thorner, P., Maxie, M. G., and Valli, V. E. O. (1991) Lab Invest. 55, 435–443.

25. Thorner, P., Baumal, R., Valli, V. E. O., and Goldberger, A. (1987) Lab Invest. 56, 955–962.

26. Thorner, P., Baumal, R., Valli, V. E. O., Mahuran, D., Mdnines, R., and Marrano, P. (1989) Kidney Int. 35, 843–850.

27. Baumal, R., Thorner, P., Valli, V. E. O., Marrano, P., Binnington, A., and Bloedow, A. (1991) Am J Pathol. 139, 751–764.

28. Zheng, K., Thorner, P. S., Marrano, P., Baumal, R., and Mdnines, R. (1994) Proc Natl Acad Sci USA 91, 3989–3993.

29. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., pp. 30–31, Academic Press, Cold Spring Harbor, NY.

30. Morrison, K. E., Gernino, G. G., and Reenders, S. T. (1991) J. Biol. Chem. 266, 34–39.
36. Neilson, E. G., Kalluri, R., Sun, M. J., Gunwar, S., Danoff, T., Mariyama, M., Myers, J. C., Reeders, S. T., Hudson, B. G. (1993) J. Biol. Chem. 268, 8402–8405
37. Morrison, K. E., Mariyama, M., Yang-Feng, T. L., and Reeders, S. T. (1991b) Am. J. Hum. Genet. 49, 545–554
38. Sugimoto, M., Oohashi, T., Yoshioka, H., Matsuo, N., and Ninomiya, Y. (1993) FEBS Lett. 330, 122–126
39. Oberbäumer, I., Laurent, M., Schwarz, U., Sakurai, Y., Yamada, Y., Vogeli, G., Vois, T., Siebold, B., Glanville, R. W., and Kühn, K. (1985) Eur. J. Biochem. 147, 212–224
40. Burbelo, P. D., Martin, G. R., Yamada, Y. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 9679–9682
41. Miner, J. H., and Sanes, J. R. (1994) J. Cell Biol. 127, 879–891
42. Yoshioka, K., Hino, S., Takemura, T., Maki, S., Wieslander, J., Takekoshi, Y., Makino, H., Kagawa, M., Sado, Y., and Kashtan, C. E. (1994) Am. J. Pathol. 144, 986–996
43. Ninomiya, Y., Kagawa, M., Iyama, K., Naito, I., Kishiro, Y., Seyer, J. M., Sugimoto, M., Oohashi, T., and Sado, Y. (1995) J. Cell Biol. 130, 1219–1229
44. Peissel, B., Geng, L., Kalluri, R., Kashtan, C., Rennke, H. G., Gallo, G. R., Yoshioka, K., Sun, M. J., Hudson, B. G., Neilson, E. G., and Zhou, J. (1995) J. Clin. Invest. 96, 4839–4844
45. Ding, J., Stitzel, J., Berry, P., Hawkins, E., and Kashtan, C. E. (1995) J. Am. Soc. Nephrol. 5, 1714–1717
46. Guo, C., Van Damme, B., Van Damme-Lombaerts, R., Van den Berghe, H., Cassiman, J.-J., and Marynen, P. (1993) Kidney Int. 44, 1316–1321
47. Zhou, J., Gregory, M. C., Hertz, J. M., Barker, D. F., Atkin, C., Spencer, E. S., and Tryggvason, K. (1993) Kidney Int. 43, 722–729
48. Lemmink, H. H., Kluftmans, L. A. J., Brunner, H. G., Schröder, C. H., Knebelmann, B., Jelinková, E., van Oost, B. A., Monnens, L. A. H., and Smeets, H. J. M. (1994) Hum. Mol. Genet. 3, 313–322
49. Spranger, J., Winterpacht, A., and Zabel, B. (1994) Eur. J. Pediatr. 153, 56–65
50. Hilaî, L., Rochat, A., Duquesnoy, P., Blanchet-Bardon, C., Wechsler, J., Martin, N., Christiano, A. M., Barrandon, Y., Uitto, J., Goossens, M., and Hovnanian, A. (1993) Nat. Genet. 3, 287–293
51. Willing, M. C., Pruchno, C. J., Atkinson, M., and Byers, P. H. (1992) Am. J. Hum. Genet. 51, 508–515
52. Stover, M. L., Primorac, D., Liu, S. C., McKinstry, M. B., and Rowe, D. W. (1994) J. Clin. Invest. 92, 1994–2002
53. Pöschl, E., Pollner, R., and Kühn, K. (1988) EMBO J. 7, 2687–2695
54. Fischer, G., Schmidt, C., Optz, J., Cully, Z., Kühn, K., and Pöschl, E. (1993) Biochem. J. 292, 687–695
55. Schmidt, C., Fischer, G., Kadner, H., Genersch, E., Kühn, K., and Pöschl, E. (1993) Biochim. Biophys. Acta 1174, 1–10
56. Heikkilä, P., Söninen, R., and Tryggvason, K. (1993) J. Biol. Chem. 268, 24677–24682
57. Burbelo, P. D., Bruggeman, L. A., Gabriel, G. C., Klotman, P. E., and Yamada, Y. (1991) J. Biol. Chem. 266, 22297–22302
58. Tanaka, S., Kaytes, P., and Kurkinen, M. (1993) J. Biol. Chem. 268, 8862–8870
59. Burbelo, P. D., Horikoshi, S., and Yamada, Y. (1990) J. Biol. Chem. 265, 4839–4843
60. Schmid, S. R., and Linder, P. (1992) Mol. Microbiol. 6, 283–292
61. Kuivaniemi, H., Tronn, G., and Prockop, D. J. (1991) FASEB J. 5, 2052–2060
62. Fischer, G., and Schmid, F. X. (1990) Biochemistry 29, 2205–2212
63. Hemmingsen, S. M., Woolford, C., van der Vies, S. M., Tilly, K., Dennis, D. T., Georgopoulos, C. P., Hendrix, R. W., and Ellis, J. (1988) Nature 333, 330–334
64. Urlaub, G., Mitchell, P. J., Ciudad, C. J., and Chasin, L. A. (1989) Mol. Cell. Biol. 9, 2868–2880
65. Sachs, A. (1993) Cell 74, 413–421