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Genetics

The congenital goiter mutation is linked to the thyroglobulin gene in the mouse

(hypothyroidism/gene mapping/mouse chromosome 15)

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ABSTRACT **Rat thyroglobulin (TG) cDNA clones were used to identify DNA restriction fragment variants among inbred mouse strains. One of these variants was shown to be closely linked to the recessive mutation congenital goiter (*cog*), which had previously been mapped to mouse chromosome 15. These results indicate that the structural gene for thyroglobulin is on chromosome 15 and suggest that a mutation at the site of the TG gene is the basis of the *cog* defect. No differences were observed between *cog/cog* and +/+ DNA in Southern blots using TG cDNA probes corresponding to 88% of the coding sequences, suggesting that the *cog* mutation is not due to a large deletion of this portion of the gene. Neither was there any obvious qualitative or quantitative difference between mutant and normal TG mRNA as judged by blot hybridization of electrophoretically fractionated thyroid RNAs. The thyroglobulin gene locus (*Tgn*) was mapped near the glutamic-pyruvic transaminase isoenzyme locus *Gpt-1*. The *Tgn* locus is syntenic with the c-*myc* protooncogene locus (*Myc*) in the mouse as in the rat and man.**

The thyroid hormones thyroxine (T4) and triiodothyronine (T3) are synthesized in a complex process from the $M_r \approx$ 660,000 thyroglobulin (TG) protein precursor (1). TG is a large glycoprotein consisting of two identical subunits whose primary polypeptide chain is of $M_r \approx 300{,}000$. Two pairs of primary hormonogenic tyrosine residues have been identified near the amino and carboxyl termini of the polypeptide (2, 3). These tyrosine residues are iodinated and coupled by ether bonds. Thyroid hormones are released when TG is endocytosed by the thyrofollicular cells from the follicular lumen, where it is stored, and degraded in the lyzosomes by proteolytic digestion. Parts of the TG gene have been cloned in several species including the cow, goat, rat, and man (3–7). The rat TG gene consists of 42 exons, spanning more than 170 kilobases (kb), and is transcribed into an ≈8500-nucleotide mRNA (5). The human TG gene is >300 kb long (6). Hereditary hypothyroidism due to disorders of TG biosynthesis has been described in both man and several species of domestic animals (1, 4, 8). Recently, a mouse mutation, congenital goiter (*cog*), was described that in homozygous condition causes primary hypothyroidism with goiter (9). We have used rat TG cDNA clones to identify and map DNA restriction fragment variants associated with the mouse TG gene. We show that the *cog* mutation is closely linked to these TG restriction fragment variants and is therefore probably a mutation in the mouse TG gene.

MATERIALS AND METHODS

Hybridization Probes. Rat TG cDNA clones pRT27.15, pRT36.1, and pRT57 were the gift of R. Di Lauro (5). These plasmid clones were labeled by random priming (10) for hybridization to DNA or RNA blots.

DNA Extraction, Southern Blotting, and Hybridization. High molecular weight DNA was extracted from mouse spleens and digested with restriction endonucleases (Bethesda Research Laboratories), and fragments were separated by agarose electrophoresis as described (11). Southern blotting onto Zetabind (AMF, Meriden, CT) nylon filters by the NaOH transfer method with prior acid depurination (12) was followed by hybridization of the blots to ^{32}P-labeled probes in 7% $NaDodSO_4$ as described (13). The final wash was at an estimated stringency of 90%. Autoradiograms of the filters were developed by exposing x-ray film with intensifying screens for 3 days at −80°C.

Extraction and Blot Hybridization of Thyroid RNA. Total RNA was extracted from pools of 3–10 mouse thyroids that had been frozen in liquid nitrogen immediately upon dissection and stored at −80°C. The glands were homogenized in guanidine hydrochloride and extracted according to a published protocol (14). Rat thyroid and mouse thymus RNAs were extracted as positive and negative controls. RNAs (5 μg per lane) were denatured by briefly heating to 70°C prior to electrophoresis in 1% agarose. RNA was transferred to nylon filters for hybridization with pRT57 rat TG cDNA probe (15).

RESULTS

Identification of DNA Restriction Fragment Length Variants. Initially we attempted to map the mouse TG gene locus (*Tgn*) by identifying DNA restriction fragment polymorphisms whose segregation could be followed in sets of recombinant inbred (RI) strains. Genomic DNAs from a panel of inbred strains, including progenitors of RI-strain sets, were digested with various restriction endonucleases, Southern blotted, and probed with several rat TG cDNA clones. The probes hybridized to multiple restriction fragments in mouse DNA, and a few of these were polymorphic. Fig. 1 illustrates the results obtained using the enzyme *Taq* I. Most inbred strains could be classified as possessing one of three haplotypes, designated Tgn^a, Tgn^b, and Tgn^c (Table 1). The most common haplotype, Tgn^b, was found in the C57BL/6J strain and 13 other strains. Four strains (A/J, AKR/J, RIIIS/J, and SWR/J) differed from Tgn^b strains with respect to two variant fragments and were assigned the haplotype designation Tgn^a. The third group of strains, including BALB/cBy, CBA/J, CE/J, and C3H/HeJ, differed from Tgn^b strains with respect to 11 different restriction fragments and was designated Tgn^c. The identities of some of these 13 restriction fragment variants detected with probe pRT57 are shown in Table 1. Additional variants were detected with various combinations of the restriction enzymes *Bam*HI, *Eco*RI, *Hin*dIII, *Pst* I, *Msp* I, *Xba* I, and *Sst* I and the three rat TG cDNA clones. Neither of the two Tgn^a variants are shared by

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Abbreviations: TG, thyroglobulin; RI, recombinant inbred; cM, centimorgan(s).

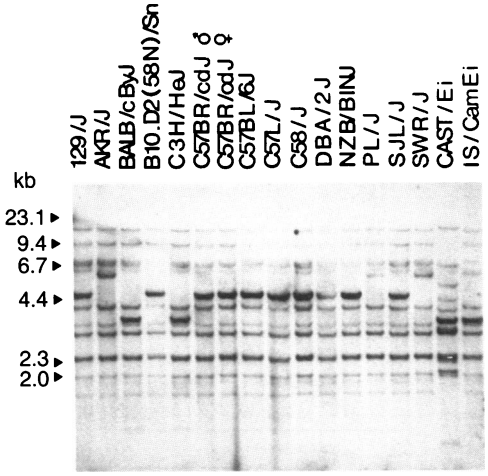

FIG. 1. Southern blot analysis of *Taq* I digests of genomic DNA (5 μg) from denoted inbred mouse strains. The blot was probed with ^{32}P-labeled rat TG cDNA (pRT57 plasmid DNA). Sizes (kb) of marker fragments (λ phage *Hin*dIII fragments) are shown at left.

Table 2. Inheritance of *Tgn* restriction fragment variants in RI strains

	RI strains	Allele
AK × D	6, 8, 9, 11, 13, 15, 18, 22, 24, 26, 27	*A*
	1, 2, 3, 7, 10, 12, 14, 16, 17, 20, 21, 23, 25, 28	*D*
AK × L	5, 24, 25, 28, 29, 37, 38	*A*
	6, 7, 8, 9, 12, 13, 14, 16, 17, 19, 21	*L*
B × H	2, 3, 4, 7, 8, 10, 11, 12, 14	*B*
	6, 9, 19	*H*
C × B	G, J	*C*
	D, E, H, I, K	*B*
C × J	1, 3, 6, 8, 9, 10, 11, 15	*C*
	4, 13	*J*
SW × L	4, 7, 12, 14, 15	*S*
	16, 17	*L*
SW × J	1, 6, 7, 10, 11, 14	*S*
	2, 3, 4, 5, 8, 9, 12, 13	*J*
L × PL	1, 4, 6	*L*
	2	*P*

DNAs from eight sets of RI strains were digested with restriction enzymes, Southern blotted, and probed with pRT57. The progenitors of the various sets of RI strains are indicated as follows: AK × D (AKR/J × DBA/2J), AK × L (AKR/J × C57L/J), B × H (C57BL/6J × C3H/HeJ), C × B (BALB/cBy × C57BL/6By), C × J (BALB/cKe × SJL/J), SW × L (SWR/J × C57L/J), SW × J (SWR/J × SJL/Wt), and L × PL (C57L/J × PL/J). Italic letters *A, B, C, D, H, J, L, P,* and *S* denote alleles inherited from progenitor strains AKR/J, C57BL/6 (J or By), BALB/c (By or Ke), DBA/2J, C3H/HeJ, SJL (J or Wt), C57L/J, PL/J, and SWR/J, respectively. The restriction enzymes used to type the various RI strains were as follows: *Pvu* II (AK × D, AK × L, SW × L, and SW × J), *Hin*dIII (B × H and C × B), *Pst* I (C × J), and *Taq* I (L × PL).

the *Tgn^c* haplotype. This distribution of restriction fragment variants is consistent with the notion that *Tgn^a* and *Tgn^c* diverged from the *Tgn^b* haplotype at different times. The segregation of *Tgn* haplotypes in eight sets of RI strains (a total of 97 strains) is presented in Table 2. Comparisons between the *Tgn* strain distribution patterns and those of numerous other genetic markers excluded linkage from many regions of the genome, but no positive association was found that would permit mapping.

Linkage Between *Tgn* and *Gpt-1*. Since the human TG gene (*TG*) had been localized to the same chromosomal band as the *MYC* protooncogene (16–18), there was reason to suspect that the mouse *Tgn* gene would be syntenic with *Myc* on mouse chromosome 15. Since linkage to the central region of chromosome 15 could not be excluded based on RI data, a backcross segregating for *Tgn* and the glutamic-pyruvic transaminase electrophoretic variant *Gpt-1* was typed (backcross 1). The results (Table 3) indicated close linkage between *Tgn* and *Gpt-1*; only one recombinant was detected among 33 backcross progeny, for an estimated recombination frequency of 0.03 (95% confidence limits, 0.0008–0.16). These results clearly place *Tgn* in the vicinity of *Gpt-1* on mouse chromosome 15.

Linkage Between *Tgn* and the Hypothyroid Congenital Goiter Mutation. Because the recently described congenital goiter mutation (*cog*) had also been assigned to chromosome 15 (9), we sought to determine whether or not *Tgn* and *cog* would be separable by recombination. Since the *cog* mutation arose on the AKR/J inbred strain (*Tgn^a*) and had been transferred subsequently to the chromosome 17 congenic AKR.L-*H-2^b* background (19), we crossed an AKR.L-*H-2^b* *cog* (hereafter referred to as the AKR.L-*cog* strain) homozygous male to the C57BL/6J-*Ve*/+ (*Tgn^b*) strain, which carries the dominant hair-texture mutation velvet (*Ve*) marking the distal part of chromosome 15. Backcrosses to AKR.L-*cog* males and intercrosses were established. Individual mice were classified at weaning as mutant (*cog*/*cog*) or normal (+/*cog* or +/+), based on size and apparent maturity. This classification was checked when the mice were killed at about 8 weeks of age, by determining thyroid weights. These two criteria usually allowed unambiguous classification of mutant

Table 1. Distribution of *Tgn* restriction fragment variants among inbred mouse strains

Haplotype*	Restriction fragment sizes,† kb			Inbred strains
	Pvu II	*Hin*dIII	*Taq* I	
a	3.0	15, 4.5, 3.0	5.5	A/J, AKR/J, RIIIS/J, SWR/J
b	2.5	15, 4.5, 3.0	4.4	BUB/BnJ, C57BL/6J, C57BR/cdJ, C57L/J, C58/J, DBA/2J, I/LnJ, LG/J, MA/MyJ, NZB/BlNJ, SF/CamEi, ST/bJ, SJL/J, 129/J
c	2.5	17, 7.5	3.5	BALB/cBy, CBA/J, CE/J, C3H/HeJ
p	2.5	15, 4.5, 3.0	5.5	PERU/AtteckEi, PL/J
—	2.5	15, 7.5	5.5	IS/CamEi
—	2.5	17, 4.5, 3.0	4.4	P/J
—	3.5, 2.5	15, 7.5	4.4	MOLF/Ei
—	4.0, 2.5	15, 7.5	5.0	CAST/Ei

*Haplotype symbols were assigned only if more than one strain shared a common restriction pattern.
†Only the variant restriction fragment sizes are given. In each digest most of the restriction fragments were shared among all strains. The listed restriction variants were detected with the pRT57 probe (see Fig. 1).

Table 3. Linkage between *cog*, *Tgn*, and other chromosome 15 markers

Backcross 1. (NZB/BlNJ × AKR/J)F_1 ♀ × NZB/BlNJ ♂			Backcross 2. (C57BL/6J-*Ve* × AKR.L-*cog*)F_1 ♀ × AKR.L-*cog* ♂					
			Ve, *cog* segregation			*cog*, *Tgn* segregation		
Gametes		No. of mice	Gametes		No. of mice	Gametes		No. of mice
Gpt-1	*Tgn*	(total, 33)	*Ve*	*cog*	(total, 58)	*cog*	*Tgn*	(total, 21)
a	*a*	16	*Ve*	+	15	+	*b*	7
b	*b*	16	+	*cog*	23	*cog*	*a*	14
a	*b*	1	*Ve*	*cog*	10	+	*a*	0
b	*a*	0	+	+	10	*cog*	*b*	0

Intercrosses. (C57BL/6J-*Ve* × AKR.L-*cog*)F_1 × (C57BL/6J-*Ve* × AKR.L-*cog*)F_1

Ve, *cog* segregation						*cog*, *Tgn* segregation		
Ve +/+ *cog* ♀ × ++/+ *cog* ♂			++/+ *cog* ♀ × *Ve* +/+ *cog* ♂					
Alleles		No. of mice	Alleles		No. of mice		No. of mice	
Ve	*cog*	(total, 244)	*Ve*	*cog*	(total, 325)	Genotype	*cog*/*cog*	+/*cog* or +/+
Ve	+	88	*Ve*	+	140	Tgn^a/Tgn^a	122	0
+	+	98	+	+	114	Tgn^a/Tgn^b	0	20
+	*cog*	43	+	*cog*	45	Tgn^b/Tgn^b	0	9
Ve	*cog*	15	*Ve*	*cog*	26			

and normal mice. In rare instances when classification was considered doubtful, the thyroid was examined histologically. The results are shown in Table 3. No crossovers were detected between *Tgn* and *cog* among 21 backcross 2 progeny. Since each *cog*/*cog* F_2 homozygote tests both maternal and paternal gametes for crossing over, we concentrated our efforts on this progeny class. All 122 F_2 *cog*/*cog* mice tested proved to be homozygous for the Tgn^a haplotype, the equivalent of no crossovers in 244 backcross progeny. One non-*cog* mouse (+/+ or +/*cog*) from each intercross mating was typed with respect to *Tgn* to verify that the F_1 parents were of the predicted *Tgn* genotype. Of the 29 non-*cog* mice typed, 9 were Tgn^b homozygotes, 20 were Tgn^b/Tgn^a heterozygotes, and none was a Tgn^a homozygote. Each Tgn^b/Tgn^a heterozygote represents an additional opportunity for recombination. In all, no crossovers were detected in 285 meiotic opportunities, consistent with allelism or close linkage between *Tgn* and *cog*. The upper 95% confidence limit of the *Tgn*–*cog* distance is 1.1 centimorgans (cM).

Loose linkage was evident between *cog* and the distal marker *Ve* (Table 3). Recombination in oogenesis was measured in backcross 2 and in the *Ve* +/+ *cog* female × + +/+ *cog* male intercross matings, while recombination in spermatogenesis was measured in the reciprocal intercross matings. Recombination frequencies were estimated by the maximum-likelihood method. Since there were no differences between the estimates obtained from the three sources, the data were pooled to provide a single combined estimate of 0.349 ± 0.031. The 95% confidence limits of this estimate are 0.288 and 0.41.

***Tgn* Restriction Patterns in Comparison of *cog*/*cog* and +/+ Genomic DNA.** Genomic DNAs from AKR.L-+/+ and *cog*/*cog* genotypes were digested with 39 different restriction enzymes, Southern blotted, and probed successively with rat TG cDNA clones pRT27.15, pRT36.1, and pRT57. Together these overlapping cDNA clones represent 88% of the rat TG mRNA. No differences between the two genotypes were detected in any of the restriction digests with any of the probes. Representative results are shown in Fig. 2. Thus, it is unlikely that the *cog* mutation involves a large insertion, deletion, or rearrangement within the parts of the *Tgn* gene detected by these probes.

Blot Hybridization Analysis of RNA Extracted from Goitrous and Normal Thyroids. Total RNA was extracted from pooled thyroids from +/+, +/*cog*, and *cog*/*cog* mice, electrophoretically fractionated, blotted, and probed with pRT57. Controls included rat thyroid RNA and mouse thymus RNA. A prominent band of hybridization was observed in each lane containing thyroid RNA (Fig. 3), in the 8- to 9-kb range corresponding to the mature TG mRNA described for the rat and other species. Each thyroid RNA showed a relatively large amount of hybridizing material that is smaller than the full-sized TG mRNA. Presumably this represents partially degraded mRNA that was either present *in vivo* or was produced during the extraction procedure. No hybridization to thymus RNA was observed, consistent with the tissue-specificity of *Tgn* gene expression. No consistent difference in either the abundance or size distribution of *Tgn*

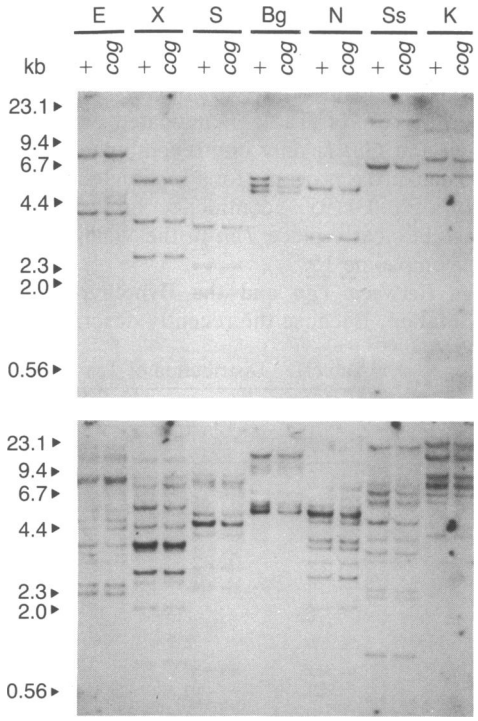

FIG. 2. Southern blot analysis of +/+ and *cog*/*cog* genomic DNA digested with *Eco*RI (E), *Xba* I (X), *Stu* I (S), *Bgl* I (Bg), *Nsi* I (N), *Sst* I (Ss), or *Kpn* I. Blot was probed with rat TG cDNA clone pRT36.1 (*Upper*) and then washed free of probe and hybridized with pRT57 (*Lower*). Size markers are shown at left as in Fig. 1.

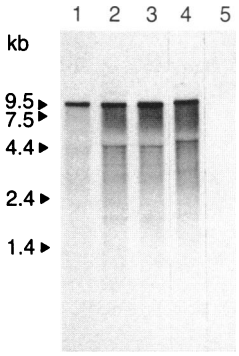

FIG. 3. Blot analysis of thyroid RNA. Rat TG cDNA clone pRT57 was ^{32}P-labeled and hybridized to size-fractionated RNA (5 μg per lane) from thyroid (lanes 1–4) or thymus (lane 5). Mouse thyroid RNAs were extracted from pools of 3–10 thyroids from genotype-matched *cog/cog* (lane 1), +/*cog* (lane 2), or +/+ (lane 3) males of the AKR.L-*H-2*^{b} background strain. Rat thyroid RNA (lane 4) was extracted from the thyroids from two strain ACI males. Thymus RNA (lane 5) was extracted from a single AKR.L-+/*cog* male mouse. Sizes and migration of a mixture of RNA size markers (Bethesda Research Laboratories) are indicated at left of autoradiograph.

transcripts was seen in any of the thyroid RNA samples. Thus, there is no apparent block of *Tgn* transcription in *cog/cog* thyroids, nor is there any evidence for a gross abnormality in the processing of *Tgn* transcripts leading to novel size classes of *Tgn* mRNAs.

DISCUSSION

TG gene DNA restriction variants were identified among inbred strains and mapped to chromosome 15. That these variants are associated with the TG structural gene (as opposed to a putative pseudogene) is supported by several considerations. First, all the restriction fragment length polymorphisms detected appear to map to one locus and are distributed as complex haplotypes among inbred strains. Second, TG cDNA probes hybridize to a similar number of fragments in digests of mouse and rat DNAs. Since there are no TG pseudogenes in the rat (5), it is unlikely that there are any in the mouse. Finally, by labeling a short region of one rat cDNA clone (corresponding approximately to rat exon 36), we were able to demonstrate hybridization to a single polymorphic restriction fragment (data not shown) that cosegregates with the *Tgn* locus. This result demonstrates a one-to-one correspondence between the *Tgn* locus and a single exon in the rat TG structural gene.

The mapping of the mouse TG gene on chromosome 15 validates a prediction based on the previous findings that the TG gene and the protooncogene c-*myc* are linked in both humans and rats (20). The human TG gene has been localized to the same chromosome band as *MYC*, 8q24. Further, the human homolog of *Gpt-1* also has been assigned provisionally to 8q (21, 22). The present finding of close linkage between the murine *Tgn* and *Gpt-1* genes lends support to this assignment. Taken together with the principle that close linkages tend to be conserved in evolution (23), the data suggest that c-*myc*, the TG gene, and the glutamic-pyruvic transaminase gene are clustered in both man and mouse. Although *Myc* has been localized to the 15 D2/3 region on the mouse cytogenetic map (24), its position in the linkage map is unknown. While *Myc* appears to occupy a proportionally more distal position on the cytogenetic map than does *Tgn* on the linkage map, this does not necessarily exclude the possibility that *Myc* is close to *Tgn* in the mouse as in man. Recombination frequency is not always proportional to physical distance in mouse chromosomes (25). In man, *TG* is known to be distal to *MYC* (16). Whether this is also true in the mouse remains to be determined.

The present mapping of *cog* and *Tgn* near *Gpt-1* presents an apparent paradox. The *cog* gene has previously been shown to be very closely linked to the dominant visible mutation hairy ears (*Eh*; ref. 9), which was in turn apparently closely linked to *Ve* (26). Yet we find only loose linkage between *Ve* and *cog*. Thus, it appears that the presence of *Eh* strongly inhibits recombination from the distal marker *Ve* to a proximal position near *Gpt-1*, a region of ≈35 cM. That *Eh* suppresses crossing-over of several distal markers has been noted by Lane and Liu (27), who hypothesized that *Eh* is closely associated with a chromosomal aberration.

The failure to detect crossovers between *Tgn* and *cog*, together with the TG-deficient phenotype of *cog/cog* mice, provides strong presumptive evidence that *cog* is a mutation in the *Tgn* gene. The 95% upper confidence limit for the *Tgn–cog* distance (1.1 cM) is <0.1% of the genome. Since *cog* is only the second mutation that causes primary hypothyroidism to be described in the mouse, the *Tgn–cog* linkage is unlikely to be coincidental. If *cog* is not a mutation at the *Tgn* locus, it is necessary to postulate that there is another gene, near *Tgn*, that controls TG accumulation posttranscriptionally. While this possibility cannot be dismissed, examples of such gene organization in eukaryotic genetics are rare.

Although the genetic evidence strongly implicates *cog* as a *Tgn* mutation, direct evidence is lacking. No differences were detected between restriction digests of +/+ and *cog/cog* DNAs using rat TG cDNA probes that encompass 88% of TG mRNA. Thus there is no indication of a sizable deletion or other rearrangement of the *Tgn* gene. Further, there is no significant difference in the size or abundance of *Tgn* transcripts in normal (+/+ and +/*cog*) or goitrous (*cog/cog*) thyroids. Since the mutant thyroid is under continuous stimulation by elevated levels of thyrotropin (9), the failure to observe a reduction of *Tgn* transcripts in the mutant thyroid cannot be interpreted to mean that the mutant and normal genes are necessarily transcribed with equal efficiency. However, it is clear that the major block to TG accumulation is not at the level of transcription or mRNA accumulation. Although the *cog/cog* thyroid contains an apparently normal-sized *Tgn* mRNA, abnormal processing of the primary transcript cannot be excluded. The excision of a small exon or the failure to excise a small intron probably would not lead to a detectable shift in the 8- to 9-kb *Tgn* mRNA band seen in blots but might drastically reduce the amount of TG synthesized.

Although the immunoreactive TG content of *cog/cog* thyroids was <1% that of normal littermates, the serum content was slightly elevated (9). This suggests that *cog/cog* thyroids synthesize an abnormal TG polypeptide, which is released into the circulation either directly or after partial degradation. One possibility is that *cog* encodes a truncated TG polypeptide chain. This possibility can be evaluated by sizing the *in vitro* translation products of *cog/cog* and +/+ TG mRNA. If a truncated TG polypeptide is found, its size might indicate the region of the *Tgn* gene likely to contain the mutation. This region could then be examined in detail to define the nature of the mutation. Another possibility is that the *cog*-encoded TG polypeptide is unstable or cannot be processed properly. In this case the genetic lesion might be more difficult to identify, but also more enlightening in regard to understanding the structural constraints of the protein.

Hereditary goiters have been reported in several species of domestic animals. The failure to synthesize appreciable amounts of TG in the case of hereditary goiters of the goat and cow have been investigated at the molecular level (4, 8). In the caprine goiter, where the goitrous thyroid contains only 0.02% of the normal level of TG antigen, a reduced amount of normal-sized TG mRNA was detected. However, no

immunologically detectable *in vitro* translation products of this mRNA were obtained. In the bovine goiter, both normal- and reduced-sized TG mRNAs were found in reduced amounts; these were translated *in vitro* into M_r 75,000 and M_r 250,000 polypeptides (8). The two mRNAs are the result of two kinds of abnormal splicing, the first leading to a frameshift and the second to the precise excision of the large ninth exon. The presence of essentially normal levels of apparently normal-sized TG mRNA in *cog/cog* thyroids distinguishes the murine congenital goiter from both the caprine and bovine models.

The identification of the *cog* mutation as a candidate for a primary defect in the *Tgn* gene has a number of implications. First, efforts to understand the biochemistry, physiology, and pathology of the mutant mouse can now be focused on the *Tgn* gene and gene product. Second, the *cog* mutant provides an excellent animal model for one class of human hypothyroidism (28). In this respect, the *cog* mouse offers obvious advantages over the large-animal models heretofore available. Third, the present results demonstrate the utility of restriction fragment variant mapping for identifying candidate mutants for primary defects at the probed locus. Although hundreds of spontaneous or induced murine mutations have been identified and mapped, the primary defect of all but a few of these is unknown. Of those with known defects, the number of these that are presently accessable to molecular analysis is even more limited. These include the genes encoding the α and β chains of hemoglobin (29–31), myelin basic protein (32, 33), malic enzyme (34, 35), and myelin proteolipid protein (36). As more mutations and cloned genes are mapped, undoubtedlly more candidate genes will be identified. Since *cog/cog* mice are viable and fertile, it would be feasible to attempt to induce and identify other mutant alleles at the *cog* locus by mating mutagenized +/+ mice to *cog/cog* homozygotes and looking for runted progeny. Such mutants might include deletions, which could be more readily associated with the *Tgn* gene. Finally, the *cog/cog* mouse could be used to test the expression of artificial *Tgn* gene constructs when introduced into transgenic mice. This approach could yield valuable information about *Tgn* regulatory elements and the functions of TG protein domains.

Direct evidence that *cog* is a mutation at the *Tgn* gene and discovery of the nature of the mutation await further studies. The fact that *cog* occurred in an inbred background means that the nature of the lesion can be determined, potentially, without the ambiguity arising from sequence polymorphism. It will be important to determine whether the TG mRNA found in *cog/cog* thyroids is translatable to a normal-sized protein *in vitro*. It may be necessary to isolate and compare *Tgn* cDNA and genomic clones from +/+ and *cog/cog* mice to completely define the genetic lesion.

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