Homeostasis in Mice with Genetically Decreased Angiotensinogen Is Primarily by anIncreased Number of Renin-producing Cells*

(Received for publication, January 22, 1999, and in revised form, March 1, 1999)

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Here we investigate the biochemical, molecular, and cellular changes directed toward blood pressure homeostasis that occur in the endocrine branch of the renin-angiotensin system of mice having one angiotensinogen gene inactivated. No compensatory up-regulation of the remaining normal allele occurs in the liver, the main tissue of angiotensinogen synthesis. No significant changes occur in expression of the genes coding for the angiotensin converting enzyme or the major pressor-mediating receptor for angiotensin, but plasma renin concentration in the mice having only one copy of the angiotensinogen gene is greater than twice wild-type. This increase is mediated primarily by a modest increase in the proportion of renal glomeruli producing renin in their juxtaglomerular apparatus and by four times wild-type numbers of renin-producing cells along afferent arterioles of the glomeruli rather than by up-regulating renin production in cells already committed to its synthesis.

An essential feature of complex organisms is the ability to maintain near constancy of their internal environments. Homeostasis is maintained by the operation of sophisticated systems that permit desirable physiological changes in biological variables, but that also act homeostatically if external factors cause undesirable changes in the variables. Genetic heterogeneity, such as is inherent to all outbred species including humans, also tends to cause variation in the internal environment. Yet the extent and types of homeostatic changes induced by naturally occurring genetic differences have not received much attention. We have recently been carrying out experiments aimed at identifying genes whose quantitative expression affects an important biological variable, blood pressure. To this end, we have used gene targeting in mice to alter the expression of the genes coding for the angiotensinogen gene (AGT) of several candidate genes, and so to produce systematic changes in their expression of the same endocrine renin-angiotensin system (RAS). The renin-angiotensin system (RAS) (1) is critical for controlling blood pressure and salt balance in mammals. Angiotensinogen (AGT) is the sole source of angiotensin II (AngII), the major active peptide of the system. AGT is synthesized primarily in the liver and is secreted constitutively into the bloodstream. It is the substrate for renin, a highly specific protease whose only known substrate is AGT. The majority of renin synthesis and secretion into the bloodstream in normal mature animals is by modified smooth muscle cells in the juxtaglomerular apparatus (JGA) of the kidney. The action of renin on AGT produces the decapetide angiotensin I (AngI), which has no significant cardiovascular activity. Angiotensin-converting enzyme, ACE, a dipeptidase present in the bloodstream as a circulating protein and in tissues as a membrane bound protein, converts AngI to the vasoactive octapeptide AngII. Genetic heterogeneity has been demonstrated at the angiotensinogen locus in humans (1, 2), and two common alleles are associated with quantitative differences in the plasma concentration of AGT and with differences in blood pressure. In previous experiments (3, 4) quantitative differences in expression in mice of the angiotensinogen gene (Agt) have been shown to directly cause modest changes in blood pressure. Here we explore the long term homeostatic adjustments that occur in mice attempting to restore their blood pressures to normal in the face of inheriting below normal expression of the angiotensinogen gene (Agt).

EXPERIMENTAL PROCEDURES

Animals—Except as indicated, all mice used were F1 hybrids between the inbred strains 129 and B6 with or without a disruptive mutation in the 129-derived copy of the Agt gene. The mutation in the Agt gene was generated in embryonic stem cells from the substrain 129/OlaHsd (5). Prior to the matings to produce the F1 hybrids, the Agt gene mutation had been maintained for several generations on the closely related substrain 129J. The mice were fed regular chow and handled following the National Institutes of Health guidelines for the care and use of experimental animals.

Protein Studies—Blood samples were rapidly withdrawn from the descending aorta of mice after exposure to an atmosphere of CO2 (less than 1 min from loss of consciousness to the end of collection). The blood was collected into ice-cold microcentrifuge tubes containing EDTA and was immediately centrifuged to isolate plasma. Plasma AGT and (active) renin concentrations were determined by radioimmunoassay as described previously (4). Plasma renin concentrations were determined after conversion to active renin by the trypsin-Sepharose 4B
To determine the area of juxtaglomerular apparatuses in the Agt one-copy and wild-type mice, 10 random fields of each section were captured with a video camera. Every section was screened using the same magnification (×400), and only the JGA with a classic donut-shaped outline were evaluated. All the images were studied with an image analysis software (Mocha™, version 1.02, Jandel Scientific). Using the manual measurement mode, the perimeter of each JGA was outlined, and its area was determined by summing the number of pixels contained within the outline.

To determine the number of cells in the JGA that had been evaluated for area, the number of nuclei observed within the outlined perimeter was counted.

Microvascular Dissection—To obtain an integrated view of the distribution of renin within the kidney, the entire renal arterial tree was dissected as described previously in rats and mice (26, 27) and stained for renin. The distribution of renin within the kidney was classified as described previously (28). In a type I distribution, renin is present along the whole length of the afferent vessel. In type II, renin extends upstream from the glomerulus but does not occupy the whole length of the vessel. In type III, renin is present as rings along the afferent vessel. In type IV, renin is restricted to the classical juxtaglomerular localization. In type V, no renin is found in the arteriole.

Statistics—All values are expressed as mean ± S.E. The two-tailed t-test was used for statistical evaluations.

RESULTS

The Experimental Animals—The experimental animals used for investigating long term homeostatic compensations in the RAS have a single functional copy of the Agt gene and one disrupted by gene targeting. We refer to them as Agt one-copy mice, and their blood pressures are about 8 mmHg (approximately 7%) below the pressures of wild-type mice with two copies of the gene (4). Except when indicated, the mice studied were F1 progeny-derived from the two inbred strains 129 and C57BL/6 (B6) and so were genetically identical except for having different numbers of functional Agt genes. Because of this genetic uniformity, even small differences between the mice can be ascribed directly to the difference in Agt gene copy number.

Renin-Angiotensin System Proteins and Peptides—To determine what components are homeostatically adjusted in the Agt one-copy animals, the steady state concentrations of the major RAS protein components present in plasma were compared in the Agt one-copy animals and their wild-type controls. The resulting data, Table I and Fig. 1 (below), show two major differences. First, the plasma AGT concentration in the Agt one-copy mice is markedly reduced, to 33–37% of the AGT concentration in the controls (p < 0.01), which is also significantly less than the 50% expected if the amount of protein were directly related to gene copy number (p < 0.01 versus 50%). A possible complication affecting this observation is that the functional Agt gene in the one-copy animals is derived from mouse strain B6, whereas the wild-type two-copy animals have one copy from strain B6 and one from strain 129. However, a comparison of AGT levels in wild-type inbred strain B6 and 129 mice shows that B6 mice have higher AGT levels (558 ± 30 AngI ng/ml/h, in six females) than do 129 mice (426 ± 11 AngI ng/ml/h, in six females), so that if strain differences in Agt gene expression persist in the F1 hybrids, the AGT concentration in the Agt one-copy animals should be even more than 50% of the wild-type F1 animals. We conclude that the plasma concentration of AGT shows no evidence of any compensatory increase in the Agt one-copy animals.

The second major difference is that, in marked contrast to the absence of any detectable compensation in AGT plasma concentration, the plasma renin concentration in the Agt one-copy animals is very significantly higher (240%) than in the wild-type controls (p < 0.001), indicating a marked homeostatic adjustment in this component of the RAS.

In mice and humans, renin is secreted into the circulation by
To investigate this possibility, we determined the plasma concentration of prorenin in one-copy and wild-type male and female mice. The results show that the prorenin levels in one-copy mice are significantly higher (p < 0.01) than in the wild-type mice. This increase in plasma prorenin is virtually identical to that of the plasma active renin, so that the same ratio of prorenin and active renin is observed in one-copy and wild-type mice. We conclude that a change in the ratio of these two products is not part of the homeostatic adjustment made in the one-copy mouse.

To determine the net effect of the observed increase in plasma renin concentration combined with the observed decrease in plasma AGT concentration, we compared the steady state concentrations of AngI in one-copy and wild-type mice. The results show that the one-copy mice have AngI levels that are 58% and 75% of wild-type in males and females, respectively. Thus the combined effect of the two changes is a partial but not complete restoration of the AngI concentrations to the wild-type level (p < 0.001 for one-copy versus wild-type), albeit at the expense of decreasing the steady state concentration of AGT below 50% of wild-type.

An additional possible means of compensating for the less than normal AGT and AngI plasma levels in the one-copy animals would be via a homeostatically induced increase in the level of the converting enzyme ACE. Measurements of serum ACE activities (Table I), however, show no significant differences (p = 0.15) between the one-copy and wild-type controls. An additional indicator of possible changes in ACE function is the plasma bradykinin concentration, since this octapeptide is destroyed by the enzyme. We found that the bradykinin levels were not different between the one-copy mice and the wild-type mice (p = 0.87). Thus we conclude that homeostatic compensation has not been induced at the level of the converting enzyme or of the bradykinin peptide.

The major effector peptide of the RAS is the octapeptide AngII. A measure of changes in the net status of the circulating arm of the system can therefore be obtained by comparing the steady state plasma concentrations of AngII in one-copy and wild-type mice. The resulting data show that plasma AngII in one-copy males and females are, respectively, 50% and 62% of the levels in the wild-type animals. These levels are significantly less than wild-type (p < 0.01), indicating that homeostasis is incomplete, as is reflected by the residual differences in blood pressure between one-copy and wild-type animals.

In summary, (Fig. 1), measurements of the expression of the protein and peptide components of the endocrine RAS show clear evidence that a major homeostatic compensation occurs in plasma renin concentrations in response to a genetic reduction in AGT gene expression. Other components of the system either show no changes or have changes that appear to be passive and secondary to the genetic reduction in AngII and the consequent homeostatic increase in renin. The final result is a steady state concentration of AngII in one-copy animals that is still significantly less than normal.

**Renin-Angiotensin System mRNAs**—To ascertain whether the changes seen in the circulating protein components of the RAS are present at the level of transcriptional products, we used an RNase protection assay to determine the amounts of the relevant mRNAs in tissues that make the largest contribution to the plasma in one-copy and wild-type animals. The major site of synthesis of AGT secreted into blood is the liver (30), which also contains the highest abundance of AGT mRNA. Fig. 2A presents the data for AGT mRNA in one-copy and wild-type male and female mice. The results show that the one-copy mice have AngII levels that are 58% and 75% of wild-type in males and females, respectively. Thus the combined effect of the two changes is a partial but not complete restoration of the AngII concentrations to the wild-type level (p < 0.001 for one-copy versus wild-type), albeit at the expense of decreasing the steady state concentration of AGT below 50% of wild-type.

**TABLE I**

| Component | Males | Females |
|-----------|-------|---------|
| AGT       | 164 ± 6 (33) | 201 ± 16 (37) |
| Renin     | 85 ± 6 (222) | 131 ± 12 (262) |
| Prorenin  | 572 ± 51 (234) | ND |
| ACE       | 360 ± 15 (90) | 251 ± 11 (102) |
| AngI      | 34 ± 4 (98) | 45 ± 3 (75) |
| AngII     | 12 ± 1 (50) | 11 ± 2 (62) |
| Bradykinin| 96 ± 2 (103) | 70 ± 2 |

*a* p < 0.01 versus wild-type.

*b* p < 0.001 versus wild-type.

*c* p = 0.15 versus wild-type.

*d* p = 0.87 versus wild-type.

**Fig. 1.** RAS proteins and peptides in one-copy mice. A, inter-relationships of the components measured in the RAS cascade. B, levels of the RAS components in one-copy mice. The bars show means ± S.E. as percentages of those in wild-type mice (two-copy). See Table I and Fig. 3 for details.
higher (data not shown), but again the difference was not significant (108% wild-type, \( p = 0.65 \)). Thus there is no evidence for significant homeostatic compensation at the level of ACE mRNA.

In the mouse, three receptors (types 1A, 1B, and 2) control the cellular and physiologic actions of AngII (22, 33). The results of administering receptor antagonists that specifically block the actions of either the type 1 or the type 2 receptors establish that blood pressure changes are chiefly executed by the type 1 receptors (34). Genetic experiments disrupting the genes coding for the type 1A receptor gene (35, 36) or the type 1B receptor gene (37, 53) show that approximately 90% of the endocrine pressor effects of AngII are via the type 1A receptor. Another possible means of homeostatic adjustments in the face of a chronic decrease in blood pressure would therefore be to increase expression of the type 1A receptor. However, comparison of the type 1A receptor gene expression by RT-PCR (Fig. 3) in Agt wild-type mice (lane 1) and the Agt one-copy mice (lane 2) revealed no detectable differences, although the same assay readily detected the decreased level of type 1A receptor mRNA in animals having only one copy of the 1A receptor gene (lane 4) in place of the normal two copies (lane 3), and showed no product in 1A receptor gene zero-copy mice (lane 5). Thus chronic homeostatic changes in expression of the type 1A angiotensin II receptor gene do not occur in the Agt one-copy animals.

**Other Tissues**—A great deal of work by many investigators has been directed toward assessing the possible autocrine/paracrine contributions to blood pressure homeostasis by RAS components synthesized in tissues other than those directly involved in the endocrine/circulatory aspects of the system (38, 39). Homeostatic changes in other tissues were therefore investigated in the Agt one-copy and wild-type animals. The data for AGT mRNA are summarized in Fig. 4A and show that, as in the liver, the AGT mRNA levels in the kidney, submandibular gland and testis of the Agt one-copy animals are close to the levels expected for animals having only a B6-derived Agt gene. Thus there is no evidence of significant homeostatic compensation at the level of Agt gene transcription in these tissues. In the brains of both males and females, the Agt one-copy animals likewise have lower amounts of AGT mRNA than wild-type animals, but the difference in the brain is less than in all other tissues. Further studies will be required to determine whether this is due to a compensatory change in Agt gene expression in the brain or to some other factors.

Fig. 4B presents the data for renin mRNA levels in various extra-renal tissues. With one exception, renin mRNA in extra-renal tissues does not differ between the Agt one-copy and wild-type animals. The exception is the adrenal gland of males, where the level of adrenal renin mRNA in Agt one-copy males
is four times that of wild-type males ($p < 0.001$). However, no difference in adrenal renin mRNA is seen between the $Agt$ one-copy and wild-type females ($p = 0.48$). Note also that $Agt$ wild-type males have approximately four times the adrenal renin mRNA of wild-type females, so that gender-related differences in renin expression are seen in the adrenal glands irrespective of their $Agt$ genotypes.

**Gender-related Effects**—Some strains of mice including 129 have a duplicated renin locus that includes a closely linked androgen-responsive renin gene ($Ren-2$) in addition to a gender-indifferent gene ($Ren-1$); male mice carrying the $Ren-2$ gene synthesize large amounts of mRNA in their submandibular glands and secrete renin into their saliva (40, 41). Other strains, including B6 (and humans), have only the $Ren-1$ gene, and so have much lower salivary gland renin mRNA levels, comparable with the levels seen in females. The $Ren-2$ gene has not been described in any species other than mouse. The F1 mice used in the present study have the single strain B6-derived $Ren-1^b$ gene and the two closely linked strain 129-derived $Ren-2$ and $Ren-1^a$ genes. We therefore carried out experiments to determine to what extent the homeostatic changes in renin expression seen in the $Agt$ one-copy animals differed by locus as well as by gender.

The relative contribution to kidney renin mRNA of the $Ren-1$ and $Ren-2$ genes was determined by a primer extension analysis. We found that in the $Agt$ one-copy females 20% of the renin mRNA was derived from the $Ren-2$ gene, which does not differ significantly from the percentage in their wild-type sisters or in a 50/50 mixture of the two female parental mRNAs. In the $Agt$ one-copy males 40% of the mRNA was from the $Ren-2$ gene, which is greater than that in their wild-type brothers (10%) or in a 50/50 mixture of the two male parental mRNAs (10%). Thus, in $Agt$ one-copy females the homeostatic increase in renin expression is mediated by the two renin genes in the same proportion as they are expressed in their wild-type sisters, but in males a greater proportion of the increase is mediated by the androgen-sensitive $Ren-2$ gene.

**Cellular Responses**—A great preponderance of evidence supports the view that the kidney is the chief source of circulating active renin and its enzymatically inactive precursor prorenin in humans and mice (42). In the normal kidney, renin is produced by modified smooth muscle cells that are associated mainly with the juxtaglomerular apparatus (JGA) and to a much lesser extent with the afferent upstream (proximal) portion of the glomerular arterioles. Several, not necessarily mutually exclusive, mechanisms could therefore mediate the chronic homeostatic elevation of kidney renin mRNA that we observe. Transcription of one or more of the renin genes could be up-regulated in the usual renin-producing cells of the kidney, or the number of these or other cells capable of synthesizing renin could be increased. To help distinguish between these various possibilities we determined by immunohistochemistry the number and distribution of renin-containing cells in the kidneys of $Agt$ one-copy animals and wild-type controls.

The immunohistochemical results are illustrated and diagrammed in **Fig. 5**. In wild-type animals the majority of renin-producing cells are confined to the juxtaglomerular end of the arterioles in the manner typical of a classical JGA (Fig. 5, A and C). This distribution of expression corresponds to the type IV pattern (28) shown diagrammatically in the bottom panel of **Fig. 5**. A much smaller proportion of wild-type glomeruli have additional renin-staining cells extending along the afferent arterioles as well as being present in the JGA (types II and III). Some wild-type glomeruli have no renin-staining cells (type V). In the $Agt$ one-copy animals (Fig. 5, B and D), there is a considerable increase in the proportion of glomeruli having renin-staining cells extending along the afferent arteriole as well as being present in the JGA (types II, III, and mixed types II/III and III/IV).

A summary of a statistical analysis of these immunohistochemical data is presented in the upper portion of **Table II**, using combined data from 12-week-old F1 $Agt$ one-copy ($n = 5$) and wild-type mice ($n = 5$) and from 10-week-old F2 litter mates having the same genotypes ($n = 5$ and 3, respectively); the F1 and F2 data were essentially indistinguishable. This analysis shows: (i) that the proportion of glomeruli having renin-staining cells in the classical JGA region is somewhat (about 25%) greater in the $Agt$ one-copy mice than in wild-type ($p < 0.05$); (ii) that in the $Agt$ one-copy mice the number of afferent arterioles with renin-staining cells upstream from the glomerulus is three times that of wild-type mice, and this is highly significant ($p < 0.0001$); (iii) that the number of renin-staining cells along the individual afferent arterioles of the $Agt$ one-copy mice is also increased significantly (1.4 times wild-type; $p < 0.05$); (iv) that the total number of renin-staining cells along the afferent arterioles of the $Agt$ one-copy mice is more than 4 times wild-type ($p < 0.0001$).

To assess the possible occurrence of a hyperplastic response in the renin-containing cells of the JGA of the $Agt$ one-copy mice, the number of renin-staining cells in a sampling of glomeruli having classic donut-shaped JGA was determined by counting nuclei within the renin-staining areas in $Agt$ one-copy mice, and this was compared with wild type using the 10-week-old F2 litter mates. No difference was observed (lower part of **Table II**, $p > 0.7$). To assess the possible occurrence of a hypertrophic response, the area of renin staining was determined for each JGA. Again no difference between the two genotypes was observed (Table II, $p > 0.3$). The sizes of the individual renin-producing cells, as judged by the area per cell, are indistinguishable in the classic JGA of the two genotypes. The intensity of renin-staining per cell was likewise not observably different in the two genotypes.
Overall these data show that the higher plasma renin levels and the greater amount of total kidney renin mRNA in the Agt one-copy animals relative to wild type is mediated by their having a somewhat greater proportion of glomeruli with renin-expressing cells in the JGA region and a severalfold greater number of renin-expressing cells along the afferent arterioles of their renal glomeruli rather than by hyperplasia or hypertrophy of JGA cells already committed to renin synthesis or by up-regulation of renin gene expression in these cells.

DISCUSSION

The main purpose of the present study was to determine the major long term adjustments directed toward homeostasis that occur in mice inheriting a precisely determined genetic variation with a blood pressure lowering tendency, namely inactivation of one copy of the Agt gene. The first finding is that in the liver, the prime site of AGT synthesis, no homeostatic up-regulation of the remaining functional Agt gene can be detected at the mRNA level. Thus the single copy of the Agt gene in the experimental animals yields essentially half the amount of mRNA achieved by the two copies in the wild-type controls. This absence of autoregulation of a normal functional gene to compensate for some unusual behavior in its homologous allele appears to be very widespread and probably universal in genes that do not code for products that act directly with their own regulatory machinery. There is a considerable body of data from previous studies showing that the expression of many genes is directly and precisely proportional to gene copy number. Epstein (43), for example, compiled from previous studies convincing evidence in support of this proportionality in humans and mice having trisomies, monosomies, and deletions involving over 40 different loci mainly coding for enzymes and plasma proteins. And we, in “gene titration” experiments with mice, have demonstrated a direct proportionality between gene copy number and expression with the genes coding for AGT (3, 4), for ACE (44) and for the natriuretic peptide receptor A (45). In none of these instances is the normal gene up-regulated in the absence of a functional homologue, while three copies of a gene produce very close to 1.5 times the amount of immediate gene product resulting from two copies, indicating a similar
abundance of down-regulation. These observations do not exclude the existence of mechanisms to up-regulate or down-regulate genes via less direct and more complex pathways, but they do exclude the general occurrence of autoregulation. The implication is that most genetic variants which affect expression will not be corrected by adjusting the transcription of either the variant gene or its nonvariant homologue.

The second finding is that the major route whereby homeostasis in the endocrine side of the RAS is attempted in the Agt one-copy animals is through an increase in plasma renin concentration mediated by a modest increase in the proportion of glomeruli with renin-producing cells in their JGA and by the presence of considerably greater numbers of renin-producing cells along the afferent glomerular arterioles in the Agt one-copy animals than in the wild-type animals, rather than by hyperplasia or hypertrophy of JGA cells already committed to renin synthesis or by an increase in their renin content. No significant changes were detected in expression of the Ace gene or of the gene coding for the type 1A AngII receptor, which mediates most of the blood pressure-related functions of the system.

Comment is required on our observation of differences between the relative expression of the Ren-1 and Ren-2 in the male but not the female Agt one-copy and wild-type mice. In assessing the relevance of these findings to the overall problem of the homeostasis of blood pressure, it is important to recollect that the androgen-responsive Ren-2 locus has been reported only in the mouse and then not in all strains. Past work by others (17, 18) has shown that the Ren-1:Ren-2 expression ratio differs markedly between different cell types in males but not in females, ranging from around 1:1 in the male kidney to 1:100 in the male salivary gland. The relative increase in Ren-2 expression seen in the kidneys of male but not the female Agt one-copy animals suggests that the additional renin-positive cells along the afferent arterioles have some features like cells in the salivary glands and so show gender differences in our F1 mice.

Comment is also required on the 4-fold greater amounts of renin mRNA observed in the adrenals of the male Agt one-copy animals relative to the expression in their wild-type brothers. At least two arguments suggest that this increase in renin expression does not represent a general mechanism for achieving homeostasis in the circulatory arm of the RAS. First, the effect is male-limited and is therefore again likely a consequence of the mouse strain-specific androgen-responsive Ren-2 gene. Second, the plasma active renin concentration in the Agt one-copy males is actually not as much increased (2.2 times wild type) as in the one-copy females (2.6 times wild type). However, we do not exclude the possibility that the increased adrenal expression of renin in the males can act on a local arm of the RAS (46), and the phenomenon merits further investigation.

Our finding that homeostasis in the genetically modified animals is accompanied by considerably greater numbers of renin-producing cells in the kidney alters the nature of subsequent questions. In place of asking how up-regulation of the renin genes in cells already producing renin is induced and executed in the Agt one-copy animals, the question becomes what mechanism leads to the observed presence of more renin-producing cells. No definitive answer is available. However, the changes seen in the normal rat kidney during the period from late gestation to early maturity and in adult kidneys exposed to experimentally lowered blood pressures suggest some interesting possibilities. Initially in the developing rat kidney (28) the afferent glomerular arterioles stain positively for renin along the whole of their lengths (type I, as illustrated in Fig. 5). Shortly after birth, type I-staining glomeruli are much less frequent, but glomeruli can be seen in which the renin staining extends far along the arterioles from the glomerulus (type II). These in turn partly disappear, and glomeruli are seen that show the classic adult pattern, with renin staining confined to the JGA (type IV), together with glomeruli showing no renin staining (type V). The type of renin distribution that is severalfold more frequent in the Agt one-copy mice than in wild type has a staining pattern very similar to the type II pattern in the rat. This similarity suggests the possibility that in the Agt one-copy mice more of the renin-producing cells initially present along the afferent arterioles persist into adult life and fewer glomeruli cease to produce renin in their JGA.

A second possibility is that additional renin-producing cells are formed in the Agt one-copy mice from non-renin-producing smooth muscle cells. This type of metaplastic/metamorphic conversion/recruitment has been described in rat kidneys exposed to blood pressures reduced by partial arterial ligation (47, 48) and in the kidneys of rats treated with high doses of ACE inhibitor (23, 49). Cell recruitment has also been demonstrated in other systems, for example during estrogen-induced synthesis of apolipoprotein II in avian hepatocytes (50), during glucose-induced synthesis of pro-insulin in purified pancreatic beta cells (51), and during thymotropin-induced formation of intracellular colloid droplets in thyroid follicular cells (52).

In conclusion, it is clear that much remains to be done if we are to understand how homeostasis is achieved in the face of quantitative genetic variations that are likely to be common in the genes which control our internal environment. The present results suggest that lifelong genetically determined disturbances may be corrected by homeostatic adjustments in the extent to which different cell populations are retained (or discarded) during development while still allowing disappearance (or reappearance) of the same cells in maturity if circumstances change. Attempts to determine the signals and mechanisms involved in genetic homeostasis should not only look for chronic up-regulation or down-regulation of immediately relevant genes, but should also look for possible shifts in the relative frequencies of the different types of cells that participate in the system.

Acknowledgments—We thank Drs. Cora-Jean Edgell and Thomas C. Coffman for helpful discussions and comments. We also thank Hui-Ying Li, Mei-Hong Lin, Jenny Lynch, and Vasantha Reddi for their technical help.

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