enough to minimize disulfide bond formation between separate dimers. The purified heterodimer was also incubated in either 2 m*M* HCl or the same redox buffer for 1.5 hours at a peptide concentration of 34 μ*M*. The redox conditions were chosen to minimize the amount of mixed disulfide (that is, between peptide and glutathione) and reduced peptide, while maximizing the amount of disulfide-bonded species. The heterodimer was originally produced by air oxidation of reduced GCN4-p1N + reduced GCN4-p1C in 5*M* GuHCl, yielding a 1:2:1 ratio of GCN4-p1N homodimer:heterodimer:GCN4-p1C homodimer, and was then purified by HPLC.

20. The detection limits of the HPLC assay are such that we could detect a heterodimer peak if it were present at 1/1000 the concentration of the homodimers. Although there might be slight differences in the propensity to form a disulfide bond in the two orientations, the Gly-Gly linker should provide enough flexibility to minimize these differences. Since no heterodimer was detected, we conclude that the parallel orientation of helices is preferred over the antiparallel orientation by at least 1000-fold.
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32. Two-dimensional COSY (correlated spectroscopy) NMR spectra of GCN4-p1 (5 m*M* in 0.15*M* NaCl, D_2O, 30°C) exhibit only one set of crosspeaks for each leucine in the molecule (T. G. Oas, E. K. O'Shea, P. S. Kim, unpublished results), indicating that the helices are symmetric within the dimer and that one orientation of the helices predominates.
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37. We thank P. Matsudaira for advice, encouragement, and discussions in all aspects of this work; T. Oas and J. Fetrow for assistance with computer graphics; T. Alber, B. Cochran, R. Hodges, T. Oas, and J. Staley for helpful discussions; and G. Fink and D. Rio for useful comments on the manuscript. The FAB-MS studies were performed at the MIT Mass Spectrometry Facility (supported by NIH). Supported in part by grants from the Lucille P. Markey Charitable Trust and the Massachusetts Division of the American Cancer Society.

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A Genetic Polymorphism in the Renin Gene of Dahl Rats Cosegregates with Blood Pressure

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Blood pressure is influenced by multiple genetic loci whose identities are largely unknown. A restriction fragment length polymorphism (RFLP) in the renin gene was found between Dahl salt-hypertension–sensitive (S) and Dahl salt-hypertension–resistant (R) rats. In an F_2 population derived from crossing S and R rats, the renin RFLP cosegregated with blood pressure. One dose of the S-rat renin allele was associated with an increment in blood pressure of approximately 10 mmHg, and two doses of this allele increased blood pressure approximately 20 mmHg. From this it can be definitively concluded that in the rat the renin gene is, or is closely linked to, one of the genes regulating blood pressure.

HUMAN ESSENTIAL HYPERTENSION is known to have a strong genetic component, and genetic selection experiments with laboratory rats (*1, 2*) and mice (*3*) have repeatedly shown that blood pressure is a polygenic trait that responds readily to genetic selection. Several strains of genetically hypertensive rats and mice along with their counterpart normotensive (control) strains have been developed. In spite of the accumulation of significant biochemical and physiological data on such strains, little attention has been paid to identifying the genes involved in blood pressure regulation.

The technique of detecting RFLPs facilitates defining areas of the genome that control blood pressure differences between strains of laboratory animals. Since most of the hypertensive and control strains of rats are inbred, strain-specific RFLPs should occur at many loci. Any genetic locus that was polymorphic in the founding population must fix one of the alleles in the homozygous state in the inbred strains, either by chance (genetic drift) or as the result of genetic selection for blood pressure differences. Because of the possibility of chance selection and fixation of contrasting alleles in contrasting inbred strains, the mere existence of an RFLP, or any other genetic polymorphism, between a hypertensive and control strain, cannot be taken as evidence that such polymorphism is a cause of the blood pressure differences between strains. As we have emphasized previously (*4*), genes that are causally related to blood pressure (or linked to genes that are causally related to blood pressure) must remain associated with blood pressure differences in genetically segregating populations derived from crosses of a hypertensive and a normotensive strain. If the gene being followed and an increment in blood pressure cannot be separated in such segregating populations, where genes are allowed to recombine at random, then this is clear evidence that the gene in question (or a closely linked gene) is causally involved in the blood pressure differences between strains.

We have developed (*5*) inbred salt-hypertension–sensitive (S) and inbred salt-hypertension–resistant (R) strains of rats from outbred selected stock obtained from L. K.

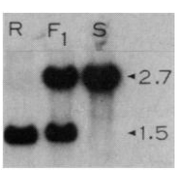

Fig. 1. Southern blot (*18*) of liver genomic DNA from Dahl salt-hypertension–resistant (R) rat, a Dahl salt-hypertension–sensitive (S) rat, and an F_1 hybrid (S × R) rat. The liver DNA was extracted by the method of Blin and Stafford (*19*), digested to completion with Bgl II restriction endonuclease, and hybridized with a radioactive nick-translated probe (*20*). The probe was a 2.7-kb Bgl II fragment from the first intron of the S-rat renin gene. The numbers at the right are in kilobases. The probe detects itself (2.7-kb fragment) in S rats and a shorter fragment (1.5 kb) in R rats; the F_1 rat shows both bands.

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Dahl (*2*). We searched these inbred strains for RFLPs using probes for the rat renin gene. Of 28 restriction enzymes tested, 8 yielded polymorphisms (*6*). Subsequently, we cloned the S and R renin genes in λ phage and determined that there are multiple differences between the S and R renin alleles (*7*). A prominent difference is a 1.2-kb insertion/deletion polymorphism in the first intron. A 2.7-kb Bgl II genomic fragment from the S renin allele that contains the insertion/deletion area serves as a convenient probe with which to type the S and R renin alleles. In Southern blots of Bgl II–digested genomic DNA, this probe detects itself in S rats (that is, a 2.7-kb band) and a 1.5-kb band in R rats; F_1 hybrids show both bands (Fig. 1).

Inbred S and inbred R strains were crossed to produce F_1 rats, then an $F_1 \times F_1$ cross was made to yield an F_2 population of 112 rats. This F_2 population was raised from weaning (4 weeks of age) on an 8% NaCl diet in order to determine the blood pressure response of each rat to high-salt intake. A scatter diagram of blood pressure responses to salt feeding of the inbred male S and R rats and the F_2 population of male rats is shown (Fig. 2). Similar blood pressure data have been obtained for females. The blood pressure data show: (i) the marked difference between S and R strains, and (ii) the wide scatter of blood pressure in F_2 rats due to the segregation of alleles at multiple loci influencing blood pressure. Blood pressure data are shown for S and R rats after 4 weeks of high-salt diet. S rats fed high-salt diet longer than this die rapidly of hypertension; R rats will live for more than a year on high-salt diet with little increase in blood pressure. Since the F_2 rats represent a wide range of genotypes at the multiple loci influencing blood pressure, it was necessary to feed high-salt diet for a longer time to F_2 rats in order to fully express the blood pressure response to salt in all rats.

The F_2 rats were killed with an overdose of pentobarbital. DNA was then extracted from livers and digested with Bgl II, and the genotype of each rat was determined by Southern blot analysis by the method shown in Fig. 1. Data on renin genotype, blood pressure, heart weight, and body weight for the F_2 population are shown (Table 1). The frequency of renin genotypes did not differ in males (12:28:19) or females 12:27:14) from the 1:2:1 ratio expected for a Mendelian trait as evaluated with the chi-square test ($P > 0.5$ in both sexes). The blood pressures among the renin genotypes were significantly different in the F_2 population. The two renin homozygous genotypes in the F_2 population differed by about 20 mmHg for males and females under the conditions of high-salt diet used here (Table 1). For both sexes the blood pressure of the heterozygotes did not differ ($P > 0.4$) significantly from the value midway between the two homozygotes as tested by Scheffé's test (*8*) for contrasts among means. Thus, each dose of the S-rat renin allele was associated with an increased blood pressure of approximately 10 mmHg. Heart weight also varied significantly with renin genotype in F_2 male rats and increased with increasing dose of the S renin allele. In females the effect of renin genotype on heart weight was less pronounced than in males (Table 1), but the reason for this is obscure.

Since blood pressure is a polygenic trait, one does not expect a priori that a given locus will account for all of the blood pressure difference between S and R rats. It has been estimated from the techniques of quantitative genetics that S and R strains differ at approximately two to four independently segregating loci that account for the blood pressure difference of the two strains (*9*).

Whether the insertion/deletion polymorphism that is the basis for the RFLP influences renin activity itself is unknown at this point. There are other structural differences between the renin alleles of S and R rats (*7*), but their functional significance relating to the renin molecule is also unknown at present. Another important unresolved question is whether the blood pressure increments segregating with the S-rat renin allele are due to biochemical or physiological effects of this renin allele or due to an allele at some other unidentified locus closely linked to the renin locus.

S rats are known to have low renin activity in kidneys, adrenals, and plasma (*10–13*) compared to R rats. Such strain differences exist before the development of overt hypertension in S rats, so that low renin activity is not necessarily just a physiological response to the higher blood pressure. S-rat adrenal (*13*) and plasma renin (*12*) is hyporespon-

Table 1. Data for blood pressure, heart weight, body weight, and number of rats classified by renin genotype for F_2 rats obtained from crossing S and R rats. Males were fed high-salt (8% NaCl) diet for 8 weeks from weaning and females for 11 weeks. In F_2 rats the blood pressure response of females to salt is slower than that of males, and thus it was necessary to feed a high-salt diet longer to females than to males in order to achieve the same blood pressure levels for the two sexes. Values are means ± SEM.

Renin genotype* by sex	*n*	Blood pressure (mmHg)	Heart weight (mg)	Body weight (g)	Heart weight/ body weight (mg/g)
Males					
Ren^r/*Ren*^{r}	12	148.7 ± 4.3	1314 ± 29	335 ± 5.0	3.93 ± 0.094
Ren^r/*Ren*^{s}	28	165.6 ± 3.5	1364 ± 20	336 ± 3.0	4.06 ± 0.063
Ren^s/*Ren*^{s}	19	171.0 ± 4.3	1438 ± 24	334 ± 7.2	4.45 ± 0.175
Probability†		0.004	0.006	0.92	0.012
Females					
Ren^r/*Ren*^{r}	12	154.4 ± 6.4	980 ± 28	235 ± 2.1	4.17 ± 0.13
Ren^r/*Ren*^{s}	27	163.9 ± 3.6	995 ± 17	230 ± 2.1	4.33 ± 0.08
Ren^s/*Ren*^{s}	14	174.8 ± 6.2	1026 ± 25	225 ± 5.0	4.59 ± 0.17
Probability		0.053	0.40	0.14	0.093

*The symbol *Ren* is used to denote the renin locus in the mouse (*17*), and it is adopted here for the rat. The R-rat allele is designated *Ren*^{r}, and the S-rat allele is designated *Ren*^{s}. †Data were analyzed for differences among genotypes by a one-way analysis of variance (ANOVA).

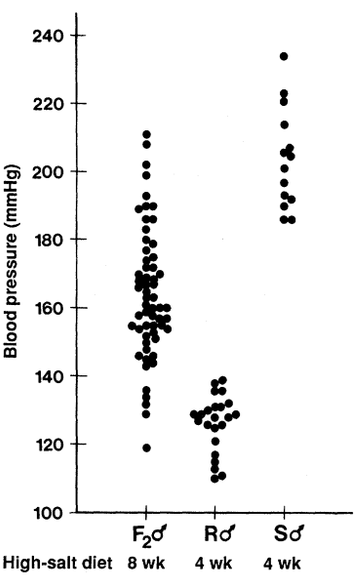

Fig. 2. Scatter diagram for blood pressures of S, R, and F_2 male rats raised from weaning on high-salt (8% NaCl) diet. Repeated blood pressure measurements (3 to 5) were made on two separate occasions (2 to 5 days apart) by the tail cuff microphonic manometer method (*21*) with the rats under light ether anesthesia after 10 min of warming at 38° to 40°C. The average measurements from the two separate sessions were averaged to obtain the final value for each rat. In the F_2 population (derived from S and R rats) genes at several loci that influence blood pressure are segregating at random; this generates a wide distribution of blood pressure in F_2 rats. Rats were handled and maintained in a humane fashion in accordance with our institutional policies and guidelines from the National Institutes of Health.

sive to a low-salt diet. The *p*H profiles for plasma renin activity differ between S and R rats (*11*), suggesting some qualitative strain difference in either renin or renin substrate.

Plasma renin originates largely from the kidneys. Since renin generates angiotensin I, which is converted into the pressor peptide angiotensin II, one expects high plasma renin activity, not low plasma renin activity, to be associated with increased blood pressure. Renin is, however, expressed in many tissues besides the kidney, including the brain (*14*), but how such nonrenal renin functions is obscure. It is also worth noting that about 25% of human essential hypertensives have low plasma renin without any obvious autonomous mineralocorticoid excess to suppress the plasma renin activity. This has led to the concept of low-renin essential hypertension (*15*).

Rats and mice have been selectively bred for high and low renal juxtaglomerular granularity. This resulted in strains with high and low renal and plasma renin levels. Blood pressures of these strains were, however, exactly the opposite of what was expected, that is, the high renin strains had lower blood pressure, and the low renin strains had higher blood pressure (*16*). These results appear compatible with the present experiments showing that the S-rat renin allele, which is presumably responsible for the low tissue and plasma renin of S rats, cosegregates with a positive increment of blood pressure.

In summary, the polymorphic renin alleles in Dahl S and R rats cosegregate with part of the blood pressure difference between these strains. This means that either the renin molecules coded by the S and R renin alleles account for the associated blood pressure differences or that alleles at some unknown locus, genetically linked to the renin locus, cause these blood pressure differences. At the very least, then, the data mean that a gene for blood pressure regulation has been localized to a part of the genome close to, or identical to, the renin locus.

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Repression of the IgH Enhancer in Teratocarcinoma Cells Associated with a Novel Octamer Factor

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Embryonal carcinoma (EC) cell lines are models for early cells in mouse embryogenesis. A 300–base pair fragment of the heavy chain enhancer was inactive in F9 EC cells, unlike in other nonlymphoid cells where it has significant activity. Alterations of the octamer motif increased enhancer activity. Nuclear extracts from F9 cells contained an octamer binding protein (NF-A3) that was unique to EC cells; the amount of NF-A3 decreased upon differentiation. It is proposed that NF-A3 represses specific regulatory sequences that contain the octamer motif. Thus, the same DNA sequence mediates either negative or positive transcriptional effects, depending on the cell type.

EMBRYONAL CARCINOMA (EC) CELL lines are derived from the stem cells of teratocarcinomas and have properties of ectodermal cells of the embryonic inner cell mass (*1*). One EC cell line, F9, when induced with retinoic acid and cyclic AMP, differentiates into extraembryonic parietal endoderm (*2*). Enhancer sequences from Simian virus 40 (SV40), polyoma virus, and Moloney murine leukemia virus (MuLV) do not function efficiently in undifferentiated F9 cells (*3, 4*). Upon differentiation, negative regulation of the viral enhancers is removed and viral expression is augmented (*3, 4*).

The 300-bp Pvu II–Eco RI fragment of the mouse immunoglobulin (Ig) heavy chain enhancer (μ300) (Fig. 1A) is active in nonlymphoid cells (*5, 6*). Located upstream of a truncated c-*fos* promoter–chloramphenicol acetyltransferase (CAT) fusion gene, the μ300 enhancer increases transcription seven times in BALB/c 3T3 cells (Fig. 1C) (*5*). The same construct bearing the wild-type μ300 enhancer in undifferentiated F9 cells was inactive in F9 cells (*7, 8*) (Fig. 1A); the CAT enzyme activity was comparable to that with no enhancer (Fig. 1, B and C).

Because the lack of activity could be due to either the absence of positive regulatory factors or the presence of negative regulatory factors, we analyzed mutations in the enhancer that abrogate binding of known trans-acting factors (*5*) (Fig. 1A). Mutations that eliminate binding of the NF-μE3 factor ($E3^-$) had no significant effect on activity (Fig. 1C). Mutations in the octamer motif ($OCTA^-$), however, increased the CAT activity more than six times (Fig. 1, B and C; O^-). To determine whether elimination of the ability to bind octamer factors was the critical effect of this mutation (as opposed to generating a novel factor-binding site), we created an 8-bp deletion of the octamer site (*9*) (Fig. 1A; DO1). Again, this increased enhancer activity (Fig. 1C).

We tested the effects of the wild-type and mutant octamer enhancers in a stable transfection assay with the use of a fusion gene, which consisted of an enhancerless MuLV promoter and the neomycin resistance (neo^r) gene (Fig. 2A) (*10, 11*). In NIH 3T3 cells, the wild-type enhancer increased the number of neo^r colonies two and one-half times in the A orientation and four times in

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