damage in some ADX animals was due to incomplete adrenalectomy or the presence of ectopic adrenal cells or other tissues that produce compounds with adrenal hormone activity.

Our finding that adrenalectomy selectively destroys granule cells is particularly unexpected because these cells are the hippocampal neurons most resistant to ischemic insult, hypoxia, or seizure activity (*3, 13*). The mechanism by which adrenalectomy causes the selective loss of hippocampal granule cells is unknown. The simplest explanation is that the granule cells need adrenal hormone receptor stimulation to remain viable. The possibility that granule cells die when their mineralocorticoid (Type I) or glucocorticoid (Type II) receptors (*4*) are not occupied by endogenous ligand is provocative. These cells may require a protein or other factor that is regulated by adrenal hormone receptor activation. Conversely, granule cell loss could be due to a process secondary to adrenalectomy, and the prevention of this loss by corticosterone may not be the result of a direct action of corticosterone within the brain.

Progress toward understanding the role of the hippocampus in normal learning and memory and in pathological conditions has been hampered by the difficulty in producing selective hippocampal lesions. Adrenalectomy may be a useful method to remove hippocampal granule cells selectively and then determine how learning, memory, and other functions are altered.

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8. Naive, ADX, and sham-operated male Long-Evans rats, weighing 150 to 174 g, were purchased from Harlan Sprague-Dawley. Animals were treated in accordance with the guidelines of the New York State Department of Health and the National Institutes of Health. In preliminary experiments, many ADX animals did not survive the 3-week period after surgery. Therefore, in this study all animals were given corticosterone (Sigma) (20 μg/ml) in the drinking solution (0.9% NaCl) starting within 1 week after surgery. After 1 week on hormone, the corticosterone concentration was halved and, after another week, halved again. After 3 weeks, corticosterone was removed from the drinking solution, and all animals survived.
9. Immediately before perfusion-fixation, 4 ml of blood was removed from the femoral vein under urethane anesthesia (1.25 g/kg, intraperitoneally). This sampling time was chosen to avoid the possibility that the loss of blood might cause ischemia that could contribute to the hippocampal damage. Corticosterone was measured in serum with a radioimmunoassay kit purchased from Radioimmunoassay Systems Laboratory, Carson, CA. All ADX animals used in this study exhibited undetectable corticosterone concentrations (less than 25 ng of serum per milliliter; $n = 52$). By comparison, sham-operated controls exhibited corticosterone concentrations within the normal stressed range (sham-operated: 313 ± 85 ng/ml, range: 205 to 410, $n = 4$; normal: 350 ± 50 ng/ml, range: 270 to 410, $n = 7$; means ± SD).
10. Rats were perfused through the heart with ice-cold 2% paraformaldehyde in 0.1*M* sodium acetate buffer, *p*H 6.5, for 3 min (no saline wash) followed by 30 min with ice-cold 2% paraformaldehyde and 0.1% glutaraldehyde in 0.1*M* sodium borate buffer, *p*H 8.5. After storage of the intact rat overnight at 4°C, the brain was removed and cut on a Vibratome. Sections 20-μm thick were mounted onto glass slides, dried, dehydrated, rehydrated, and stained with 0.75% cresyl violet. Alternate 50-μm sections of each brain were stained immunocytochemically (*11*).
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14. ADX rats that exhibited granule cell loss (ADX-loss) weighed less at the end of the experiment than undamaged ADX rats (ADX–no loss); ADX-loss: 409 ± 15 g, $n = 10$; ADX–no loss: 496 ± 15 g, $n = 10$; mean ± SEM, $P < 0.005$. Serum sodium and potassium concentrations were as follows. Sodium: ADX-loss, 136 ± 2 mmol/liter, $n = 6$; ADX–no loss: 145 ± 2 mmol/liter, $n = 3$; sham-operated: 145 ± 1 mmol/liter, $n = 4$; normal: 144 ± 1 mmol/liter, $n = 4$; $P < 0.001$, two-tailed t test; mean ± SD. Potassium: ADX-loss: 7.7 ± 0.5 mmol/liter, $n = 6$; ADX–no loss: 5.5 ± 0.7 mmol/liter, $n = 3$; sham-operated: 5.0 ± 0.4 mmol/liter, $n = 4$; normal: 5.0 ± 0.2 mmol/liter, $n = 4$; $P < 0.001$). Sodium and potassium were measured with a Technicon RA-1000 system.
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16. Corticosterone-treated ADX animals exhibited normal sodium and potassium concentrations (sodium, 143 ± 3 mmol/liter; potassium, 5.6 ± 0.6 mmol/liter, $n = 4$). One to 2 hours after drinking, detectable serum corticosterone was present (53 ± 21 ng/ml, $n = 6$).

17. We thank D. W. Dempster, J. H. Goodman, R. Lindsay, S. C. Rubin, R. K. S. Wong, and E. A. Zimmerman for useful discussions and criticism of the manuscript and M. Mukhtar of the Helen Hayes Hospital Clinical Laboratory for measuring sodium and potassium concentrations. Antisera raised in rabbits against CaBP or parvalbumin were generously provided by J. W. Pike and K. G. Baimbridge, respectively. A. J. Malcolm of the MRC Regulatory Peptide Group, Vancouver, Canada, provided monoclonal somatostatin antibody. Supported by an equipment gift from the Helen Hayes Hospital Citizen's Advisory Council and by NIH grant NS18201.

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Evidence That the Leucine Zipper Is a Coiled Coil

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Recently, a hypothetical structure called a leucine zipper was proposed that defines a new class of DNA binding proteins. The common feature of these proteins is a region spanning approximately 30 amino acids that contains a periodic repeat of leucines every seven residues. A peptide corresponding to the leucine zipper region of the yeast transcriptional activator GCN4 was synthesized and characterized. This peptide associates in the micromolar concentration range to form a very stable dimer of α helices with a parallel orientation. Although some features of the leucine zipper model are supported by our experimental data, the peptide has the characteristics of a coiled coil.

THE ESSENTIAL FEATURES OF THE proposed leucine zipper (*1*) are that the region containing the leucine repeat has a helical structure, that the leucines align along one face of the helix, and that interdigitation of the leucine side chains facilitates dimerization. The leucine repeat is found in several biologically interesting proteins (*1*) including two transcriptional regulators (yeast GCN4 and mammalian C/EBP) and three nuclear transforming proteins (Jun, Fos, and Myc). These DNA binding proteins do not contain recognizable helix-turn-helix (*2*) or zinc-finger (*3*) motifs. A 30-residue region immediately adjacent to the leucine repeat is required for specific DNA binding in both GCN4 (*4*) and C/EBP (*5*), suggesting that the proposed leucine zipper may have a fundamental role in arranging the DNA binding surface of these proteins (*1*).

We tested the leucine zipper model and began structural studies of these proteins by focusing on the leucine repeat itself. GCN4,

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Ac-RMKQLEDKVEELLSKNYHLENEVARLKKLVGER — GCN4-p1

Ac-CGGRMKQLEDKVEELLSKNYHLENEVARLKKLVGER — GCN4-p1N

Ac-RMKQLEDKVEELLSKNYHLENEVARLKKLVGERGGC — GCN4-p1C

Fig. 1. Sequences of the three peptides studied (*10*). The leucines that define the proposed leucine zipper structure are underlined. All of the peptides were acetylated at the NH_2-terminus. Peptide GCN4-p1 consists of the 33 residues at the COOH-terminus of GCN4. Peptide GCN4-p1N consists of the same 33 residues preceded by the residues Cys-Gly-Gly. Peptide GCN4-p1C consists of the same 33 residues followed by the residues Gly-Gly-Cys. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; G, Gly; H, His; K, Lys; L, Leu; M, Met; N, Asn; Q, Gln; R, Arg; S, Ser; V, Val; and Y, Tyr.

a positive transcriptional regulator (*6*), was chosen because it is well characterized both biochemically and genetically. The leucine repeat of GCN4 occurs in the last 30 residues of the protein. Mutational analysis has shown that the last 60 residues of GCN4 are sufficient for specific DNA binding activity (*4*). Deletion of the last 42 residues from GCN4 abolishes regulation in vivo (*7*) and DNA binding activity in vitro (*8*). Biochemical analysis indicates that GCN4 binds DNA as a dimer, and that dimers are formed in the absence of DNA (*9*).

In order to investigate the role of the leucine repeat in GCN4, a peptide corresponding to the 33 amino acids at the COOH-terminus of the protein (GCN4-p1, Fig. 1) was synthesized with solid-phase methods (*10*). The identity of the purified peptide was confirmed by mass spectrometry. The NH_2-terminus of the peptide was acetylated to avoid introducing additional charge interactions, and the COOH-terminus was left free as in the native protein. Circular dichroism (CD) spectra of GCN4-p1 indicate that the peptide is helical in physiological aqueous solution (Fig. 2A). The double minima at 208 and 222 nm and the maximum at 195 nm (Fig. 2A) are characteristic of α helices. The CD intensity at 222 nm indicates that folded GCN4-p1 is essentially 100% α helical (*11, 12*).

The helical structure of GCN4-p1 is remarkably stable for a peptide of this size. The midpoint of the temperature dependence (T_m) of the CD signal is 57°C (Fig. 2B). This T_m value depends on peptide concentration (Fig. 3), which suggests that GCN4-p1 self-associates. Sedimentation equilibrium experiments indicate that GCN4-p1 exists as a dimer in the concentration range of 10 μ*M* to 2 m*M* (*13*). We conclude that the concentration dependence of the T_m for GCN4-p1 results from a monomer-to-dimer equilibrium, and that the dimer is very stable.

The high aqueous solubility of GCN4-p1 (>20 mg/ml) suggests that hydrophobic side chains are shielded from solvent in a closely packed interface between the two helices. An interhelical crossover angle of ~20° allows side chains in a helix pair to pack efficiently in a "knobs into holes" (*14*) or "ridges into grooves" (*15*) manner. The orientation of the helices (parallel or antiparallel) has implications for the manner by which DNA is recognized.

We have used disulfide bond formation between cysteines placed at the ends of the helices as a probe of helix orientation [compare with (*16*)]. Two additional peptides were synthesized, one containing an NH_2-terminal cysteine (GCN4-p1N) and another containing a COOH-terminal cysteine (GCN4-p1C). In each case, two glycine residues were added between the helix and the cysteine residue to provide flexibility for disulfide bond formation (Fig. 1). In a mixture of GCN4-p1N and GCN4-p1C, oxidative disulfide formation favors homodimers if the orientation is parallel and heterodimers if the orientation is antiparallel.

Our results with disulfide bond formation indicate that the orientation of the helices is parallel, and that the folded conformation of the peptide is a dimer of helices: (i) Under physiological conditions, it is easy to obtain homodimers with GCN4-p1N or GCN4-p1C, but it is difficult to obtain heterodimers between the same peptides. In contrast, cysteine oxidation in the presence of a denaturant that unfolds the peptides (guanidine hydrochloride, GuHCl) yields both heterodimers and homodimers. (ii) The disulfide-bonded homodimers of GCN4-p1N and GCN4-p1C have T_m values that are independent of peptide concentration (88° and 78°C, respectively), presumably because the dimer is held together covalently (Fig. 3). When the disulfide bond in either homodimer is reduced, stability is concentration-dependent (*17*). (iii) In contrast to the homodimers, the stability of the disulfide-bonded heterodimer is concentration-dependent; the T_m is 59°C at a peptide concentration of 0.5 μ*M* (*18*).

The exclusivity of the parallel orientation can be evaluated from the equilibrium ratio of disulfide-bonded heterodimer to homodimers present in mixtures of GCN4-p1N and GCN4-p1C (*19*). If the orientation of helices is strictly parallel, then heterodimers are not expected to form disulfide bonds, because the cysteines would be on opposite ends of the heterodimer. No heterodimer is detected when a mixture of both peptides is incubated in a redox buffer (Fig. 4A). To verify that equilibrium had been reached, disulfide-bonded heterodimer (produced by oxidation in a GuHCl solution) was placed in the redox buffer (Fig. 4B). Again, there is no detectable heterodimer present at equilibrium. Based on the detection limits of our high-performance liquid chromatography (HPLC) assay, we estimate that the parallel orientation of helices is preferred over the antiparallel orientation by at least 1000-fold (*20*).

Landschulz *et al.* (*1*) proposed that the leucine zipper is a stable dimer of α helices. They argued that an antiparallel orientation of the helices would allow the macrodipole moment (*21*) of one helix to interact favorably with the matching helix, and that side chain interlocking would be favored in an antiparallel arrangement [because amino acid side chains in helices are often arranged at an angle pointing toward the NH_2-terminus (*22*)].

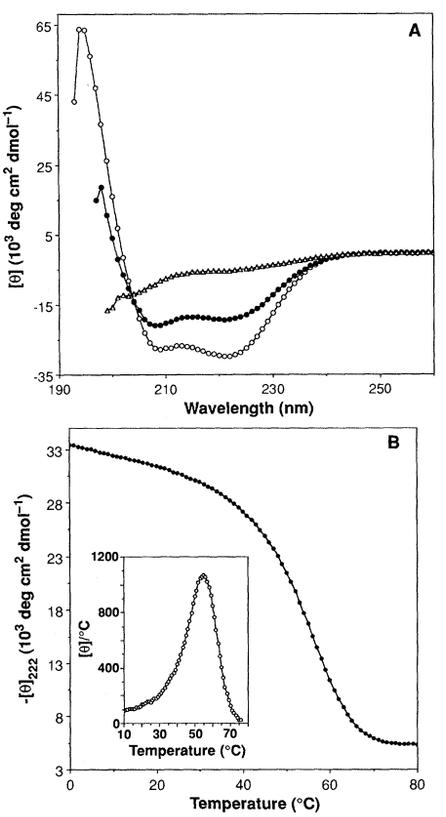

Fig. 2. (**A**) CD spectra of GCN4-p1 at (○) 0°, (●) 50°, (△) and 75°C (*17*). The value for $[\theta]_{222}$ at 0°C of −33,000 deg cm^2 dmol^{-1} indicates that GCN4-p1 is ~100% helical under these conditions (*11, 12*). (**B**) Temperature dependence of the CD signal at 222 nm for GCN4-p1 (*17*). The T_m (obtained by taking a first derivative, shown in the inset) for GCN4-p1 at a peptide concentration of 34 μ*M* was 57°C.

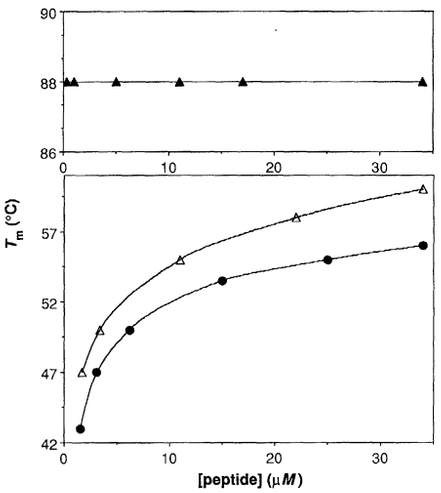

Fig. 3. The disulfide-bonded homodimers have stabilities that are independent of peptide concentration, but the stabilities of the reduced peptides and GCN4-p1 are concentration-dependent. Data are shown for (○) GCN4-p1, (△) reduced GCN4-p1N, and (▲) disulfide-bonded GCN4-p1N homodimer. Other results are given in (*17*) or in text.

Our results indicate that a peptide containing an isolated leucine zipper motif can form a very stable dimer in the micromolar concentration range. Thus, some features of the proposed leucine zipper model are supported by our experimental data. Specifically, the region containing the leucine repeats is helical and it forms a stable dimer. Our results indicate, however, that the helices of the GCN4 peptide are oriented in a parallel manner.

The leucine repeat from GCN4 has the characteristics of the coiled coil. Coiled coils are often found in fibrous proteins such as tropomyosin and keratin (*23*) and result from the coiling of two right-handed helices around one another with a slight left-handed superhelical twist (*14, 24*). The helices in most coiled coils are arranged in a parallel manner [(*25*); see however (*26*)]. The helical repeat in a coiled coil is reduced from 3.6 residues per turn (found in a classic α helix) to 3.5 residues per turn, so that the interaction pattern repeats integrally every seven residues. The leucine repeat occurs every seven residues in GCN4 and other "leucine zipper" proteins. Coiled coils also have a characteristic hydrophobic repeat interspersed within the heptad repeat (4-3 repeat) (*23*). The leucine repeat regions from GCN4, Jun, and C/EBP contain this 4-3 repeat of hydrophobic amino acids (discussed below). Although a detailed nuclear magnetic resonance (NMR) or crystallographic study is needed, we propose that a working model for the structure of at least some regions containing this motif is that of a coiled coil (*27*).

If this structure is a coiled coil, then the leucine side chains are not interdigitated like teeth in a zipper, as originally proposed [compare with figure 5a of (*1*)]. In a coiled-coil representation of the sequence from GCN4 (Fig. 5) the leucine of one helix (position 1d, 1d′) interacts extensively with the alternate, non-leucine hydrophobic residue in the opposing helix (position 1a, 1a′). Landschulz *et al.* (*1*) distinguished their hypothetical motif from the coiled coil because: (i) the interaction surface in leucine zippers is short compared with that for many naturally occurring coiled-coil proteins; and (ii) the leucine zipper structure appears to rely almost exclusively on leucine side chains, whereas coiled coils use almost any hydrophobic amino acid. We note that short segments from coiled coils, containing only four or five heptad repeats, can have substantial stability as dimers (*16*), and, although many types of residues are found in the hydrophobic positions of coiled coils, there is a strong preference for leucine (*23*).

The parallel orientation of the helices within the dimer places constraints on models for DNA binding by these proteins. For GCN4, the addition of 30 residues located at the NH_2-terminal to the region studied here is sufficient to allow specific DNA binding (*4*). The parallel, dimeric nature of the leucine repeat region may be an essential component of bidirectional transcriptional activation (*28*) and palindromic DNA binding (*29*) for GCN4. However, Myc (*30*) and some other proteins containing leucine repeats (*31*) contain a putative DNA-contact region that is far from the leucine repeat. It is possible that in some proteins the leucine repeat simply facilitates dimerization and has little to do with positioning a DNA-binding region.

A computer search identifies many proteins that contain four repeats of leucine spaced seven residues apart (*31*). Since leucine is a common amino acid, however, some of these leucine repeats may not represent true dimerization sites. Moreover, in some of these sequences, proline residues are found within the leucine repeats. Our finding that a peptide with the leucine repeat from GCN4 can, by itself, form a stable dimeric structure suggests that genetic and biochemical analyses will be useful for distinguishing leucine repeats that function as dimerization sites.

Detailed structural studies of the leucine repeat motif will be simplified using peptides, because complications involved in studying the intact protein are avoided. Indeed, preliminary analysis of GCN4-p1 indicates that high-resolution two-dimensional NMR data can be obtained, and these data confirm the conclusion that only one orientation of the helices predominates (*32*).

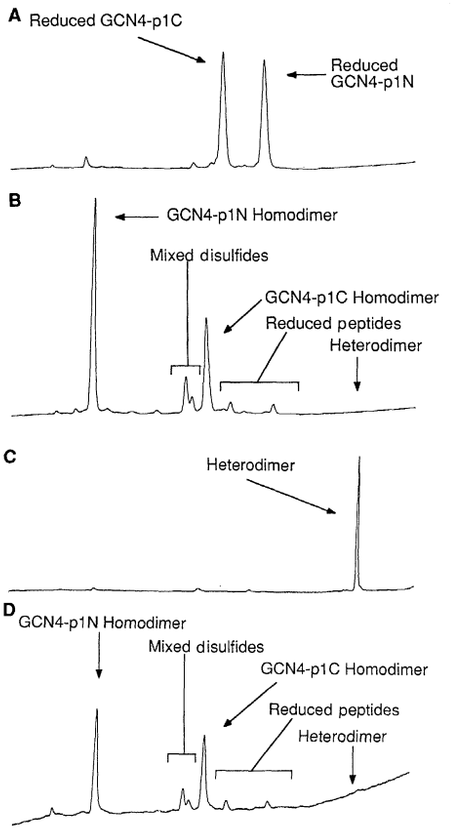

Fig. 4. High-performance liquid chromatography (HPLC) analyses of reduced GCN4-p1N plus reduced GCN4-p1C (**A**) in 2 m*M* HCl and (**B**) in redox buffer. In redox buffer (in which reshuffling of disulfides can occur), the reduced peptides equilibrated to form mostly disulfide-bonded homodimers. No detectable heterodimer was present at equilibrium, indicating that the homodimers with a parallel orientation of helices are the most stable species. A small amount of mixed disulfide species is formed by disulfide bond formation between the peptides and glutathione (the redox reagent). HPLC analyses of the disulfide-bonded heterodimer (**C**) in 2 m*M* HCl and (**D**) in redox buffer (*19*). The heterodimer equilibrated to give mostly disulfide-bonded homodimers, with no detectable heterodimer (*19*), which confirms that the reaction mixtures were indeed at equilibrium.

Finally, our finding that a synthetic peptide of a leucine repeat region forms a stable, dimeric structure could have practical applications. Such peptides may be useful as specific inhibitors of biological activity in leucine repeat–containing proteins, both in vitro and possibly in vivo. The interesting suggestion has been made (*1*) that this structure may be important in the formation of heterotypic complexes [such as between the Fos and Jun gene products; see (*33*)]. Synthetic peptides offer a way to test directly such suggestions. For both homodimer and possible heterodimer formation, it may be possible to use peptides to investigate protein-protein interactions mediated by leu-

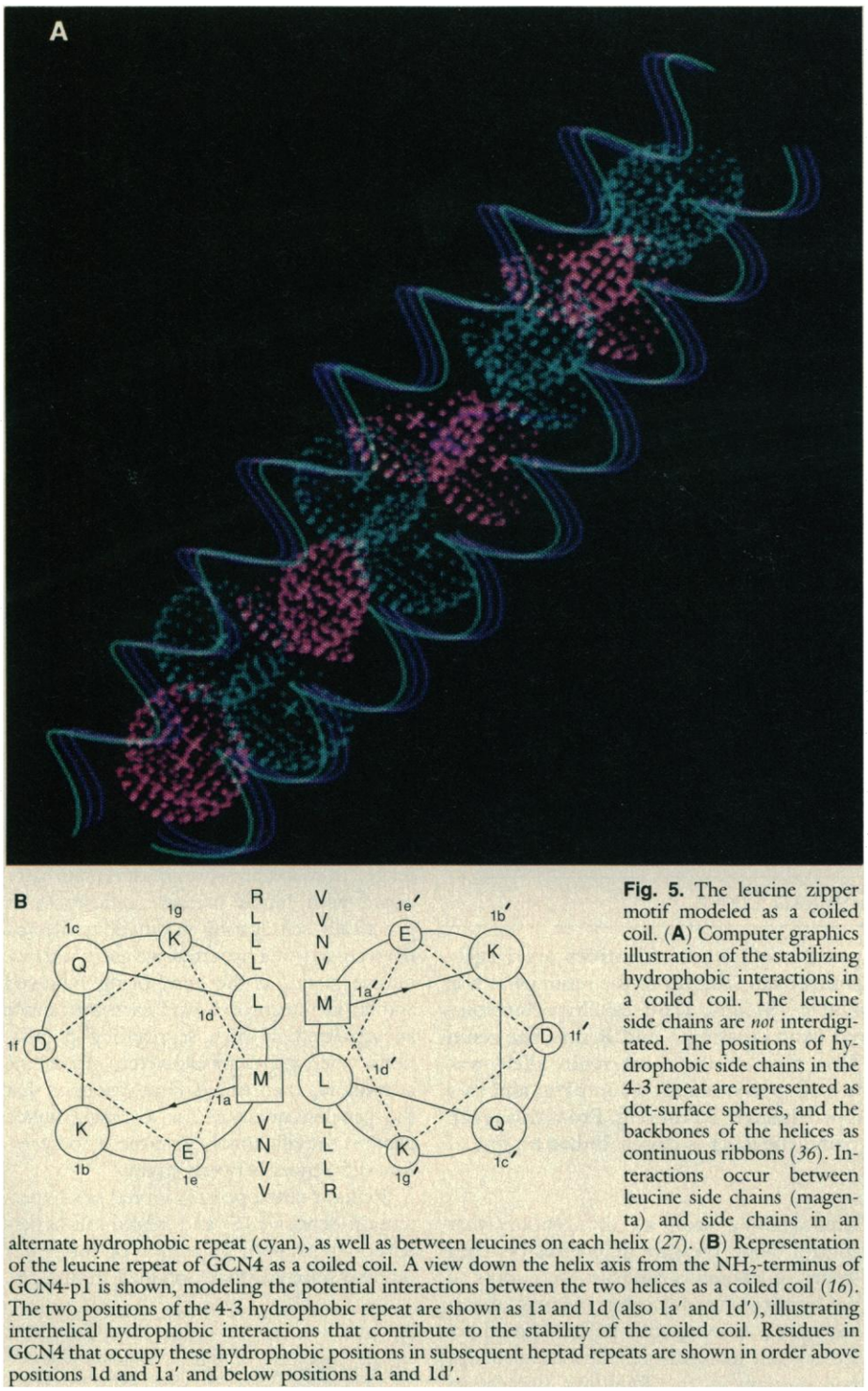

Fig. 5. The leucine zipper motif modeled as a coiled coil. (**A**) Computer graphics illustration of the stabilizing hydrophobic interactions in a coiled coil. The leucine side chains are *not* interdigitated. The positions of hydrophobic side chains in the 4-3 repeat are represented as dot-surface spheres, and the backbones of the helices as continuous ribbons (*36*). Interactions occur between leucine side chains (magenta) and side chains in an alternate hydrophobic repeat (cyan), as well as between leucines on each helix (*27*). (**B**) Representation of the leucine repeat of GCN4 as a coiled coil. A view down the helix axis from the NH_2-terminus of GCN4-p1 is shown, modeling the potential interactions between the two helices as a coiled coil (*16*). The two positions of the 4-3 hydrophobic repeat are shown as 1a and 1d (also 1a′ and 1d′), illustrating interhelical hydrophobic interactions that contribute to the stability of the coiled coil. Residues in GCN4 that occupy these hydrophobic positions in subsequent heptad repeats are shown in order above positions 1d and 1a′ and below positions 1a and 1d′.

cine repeats with affinity chromatography, protein gel overlay (*34*), and colony screening methods (*35*).

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17. The CD spectra were recorded in a 1-mm pathlength cell at a sample concentration of 34 μ*M* in PBS. All peptide concentrations were determined by tyrosine absorbance at 275.5 nm in 6*M* GuHCl [H. Edelhoch, *Biochemistry* **6**, 1948 (1967)]. Thermal unfolding studies were performed in PBS with a 1-cm pathlength cell. An Aviv Model 60DS CD spectrophotometer with an HP Model 89100A Peltier temperature control unit was used. GCN4-p1, reduced GCN4-p1N, and reduced GCN4-p1C all show a similar dependence of the T_m on peptide concentration, measured from 800 n*M* to 34 μ*M* [reduced peptides were studied in the presence of 0.5 m*M* reduced dithiothreitol (DTT) and 1 m*M* EDTA]. At 800 n*M* peptide concentration in PBS, the T_m values were: GCN4-p1, 43°C; reduced GCN4-p1N, 47°C; and reduced GCN4-p1C, 40°C. The homodimers were produced in 50 m*M* oxidized DTT, 0.1*M* tris, *p*H 8.5, followed by HPLC purification. In addition, the GCN4-p1N homodimer was thermally denatured in 4.5*M* GuHCl (near the midpoint of the GuHCl denaturation curve). These conditions were chosen to give the most sensitive test for a concentration dependence of the T_m. The T_m of GCN4-p1N homodimer in 4.5*M* GuHCl, PBS was 34° ± 0.5°C over the same range of peptide concentrations.
18. The concentration-dependent stability of the disulfide-bonded heterodimer can be explained by an association to form a dimer of heterodimers (molecular weight 17,000). This explanation is compatible with the proposed parallel orientation and also accounts for the greater stability of the heterodimer compared with GCN4-p1 at an equivalent concentration; the dimer of heterodimers has twice as many possible stabilizing interhelical interactions as does GCN4-p1.
19. The reduced peptides were mixed and incubated in either 2 m*M* HCl (control) or redox buffer (1 m*M* oxidized glutathione, 1 m*M* reduced glutathione, 0.2*M* KCl, 0.1*M* tris, 1 m*M* EDTA, *p*H 8.7) for 1.5 hours at a total peptide concentration of 34 μ*M*, and then analyzed by HPLC [an acetonitrile-H_2O gradient (with segments of 0.5% and 0.1% by volume increase per minute of acetonitrile) was used in the presence of 0.1% TFA with an analytical Vydac C-18 column]. The peptide concentration was high enough to ensure that the reduced peptides were predominantly dimers (Fig. 3), but low

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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27. Note that not all of the proposed leucine zippers contain the 4-3 hydrophobic repeat. For example, the leucine repeats from Myc and Fos contain charged residues at the alternate site. These leucine repeats may have a different conformation or alternatively, the coiled-coil structure may still form with the methylene groups of these side chains providing hydrophobic contacts, as is thought to occur in myosin [A. D. McLachlan and J. Karn, *J. Mol. Biol.* **164**, 605 (1983)].
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31. A search for additional proteins containing the leucine repeat motif yielded ~200 proteins that contain four consecutive leucine repeats spaced seven residues apart (E. K. O'Shea, J. P. Staley, P. S. Kim, unpublished results). The search was carried out with the Protein Sequence Query program to search the National Biomedical Research Foundation (NBRF) protein database.
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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36. This model was generated with coiled-coil coordinates [A. D. McLachlan, *J. Mol. Biol.* **121**, 493 (1978)] and the program INSIGHT operating on an Evans and Sutherland PS390 graphics system. Spheres that were 1.75 times the van der Waals radius were placed around the β-carbon of each hydrophobic residue of the 4-3 repeat.
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.

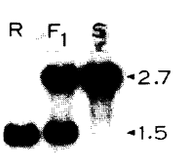

Fig. 1. Southern blot (*18*) of liver genomic DNA from Dahl salt-hypertension–resistant (R) rat, a Dahl salt-hypertension–sensitive (S) rat, and an F_1 hybrid (S × R) rat. The liver DNA was extracted by the method of Blin and Stafford (*19*), digested to completion with Bgl II restriction endonuclease, 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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