Genetic Analysis of Inherited Hypertension in the Rat

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I. Blood Pressure as a Quantitative Trait 135
II. Animal Models of Hypertension 136
III. Genomic Resources 139
IV. Strategies for Defining Quantitative Trait Loci for Blood Pressure 140
   A. Linkage analysis in segregating populations 140
   B. Recombinant inbred strains 145
   C. Congenic strains 147
   D. Other strategies for QTL analysis 149
V. Results of Quantitative Trait Loci Analysis of Blood Pressure 151
   A. Historical perspectives 151
   B. Results by chromosome 155
VI. Sex Effects on Blood Pressure Quantitative Trait Loci 161
VII. Interactions Among Blood Pressure Quantitative Trait Loci 161
VIII. Quantitative Trait Loci for Traits Related to Blood Pressure 162
   A. Cardiac hypertrophy 162
   B. Stroke 162
   C. Renal damage 163
IX. Perspective 163

Rapp, John R. Genetic Analysis of Inherited Hypertension in the Rat. Physiol. Rev. 80: 135–172, 2000.—Blood pressure is a quantitative trait that has a strong genetic component in humans and rats. Several selectively bred strains of rats with divergent blood pressures serve as an animal model for genetic dissection of the causes of inherited hypertension. The goal is to identify the genetic loci controlling blood pressure, i.e., the so-called quantitative trait loci (QTL). The theoretical basis for such genetic dissection and recent progress in understanding genetic hypertension are reviewed. The usual paradigm is to produce segregating populations derived from a hypertensive and normotensive strain and to seek linkage of blood pressure to genetic markers using recently developed statistical techniques for QTL analysis. This has yielded candidate QTL regions on almost every rat chromosome, and also some interactions between QTL have been defined. These statistically defined QTL regions are much too large to practice positional cloning to identify the genes involved. Most investigators are, therefore, fine mapping the QTL using congenic strains to substitute small segments of chromosome from one strain into another. Although impressive progress has been made, this process is slow due to the extensive breeding that is required. At this point, no blood pressure QTL have met stringent criteria for identification, but this should be an attainable goal given the recently developed genomic resources for the rat. Similar experiments are ongoing to look for genes that influence cardiac hypertrophy, stroke, and renal failure and that are independent of the genes for hypertension.

I. BLOOD PRESSURE AS A QUANTITATIVE TRAIT

Quantitative traits are those that show continuous variation from low to high values. This natural variation is present in all populations of eukaryotes and is caused by environmental or genetic factors, or by both genes and the environment. A recent short review emphasizes the interaction between genes and environment in hypertension (75). The basis for the genetic component of quantitative traits is often due to the cumulative effects on the phenotype of many genetic loci, i.e., the trait is polygenic. The loci that control a quantitative trait have been termed quantitative trait loci (QTL). The term QTL is used to describe a broad chromosomal region that may contain one or more loci controlling the quantitative trait and also to refer to one of the individual specific genetic loci participating in controlling the trait.

Many traits of biological, medical, and agricultural importance are quantitative in nature, e.g., intelligence,
body weight, body length, plant and animal disease resistance, crop yields, and seed oil content. Until the advent of molecular biology and the development of large numbers of easily typeable genetic markers, the dissection of quantitative traits into their individual genetic components was impossible except in model organisms such as Drosophila that have unique genetic properties.

Blood pressure (BP) in humans is well known to have a genetic component based on studies in families, twins, and adopted children as reviewed by Ward (266). In animals, selective breeding (reviewed below for rodents) for high or low BP from outbred stock proves that genes controlling variation in BP segregate in essentially all such stocks. Blood pressure at the high end of the distribution (hypertension) predisposes humans (and rats) to cardiovascular disease (stroke, coronary heart disease, heart failure, peripheral vascular disease, and renal failure), and the higher the BP, the worse the cardiovascular disease (154). High BP above 140 mmHg systolic or 90 mmHg diastolic is generally defined as hypertension; this classification, although useful, is an arbitrary truncation of a continuous distribution. Because genetic hypertension (so-called essential hypertension) has a high prevalence that increases with age in Westernized populations (271), it represents a major health problem. For these reasons, it is desirable to describe the genes that account for the natural variation of BP in populations as a requisite for understanding the genetic causes of hypertension.

The physiological systems altering BP are well known, and there are potent pharmacological agents for lowering or increasing BP designed around these known physiological systems. More recently, transgenic experiments in mice allow deletion or addition of specific genes that influence BP (109, 168, 177, 235, 236, 237). Such experimental manipulations of physiological systems are very informative on a physiological level because they provide information on how quantitative variation in candidate genes influences BP in the context of the whole animal with all its regulatory and compensatory systems intact. It needs to be emphasized, however, that such manipulations (with one possible exception, Ref. 84) give no evidence as to which genetic loci actually do harbor naturally occurring alleles that are present in populations in high enough frequency to account for the high prevalence of hypertension. Because this review deals with detecting naturally occurring alleles altering BP, detailed review of the transgenic literature on candidate genes is not included. It is noted, however, that once naturally occurring candidate allelic variation is detected, the ultimate test of its BP effect will almost certainly be transgenic experiments to substitute very specific DNA variation into endogenous genes by homologous recombination to evaluate their BP effects in vivo. Such experiments have not been done in the rat, although this may be feasible in the future (see sect. IX).

Although BP behaves in populations as a polygenic quantitative trait, several specific syndromes increasing or decreasing BP have been recognized in humans (19, 76, 141, 142, 216, 223, 230, 232). These are caused by rare mutants that result in drastic physiological perturbations that are penetrant enough to be observable as Mendelian traits segregating in human families. Such syndromes are usually associated with large quantitative effects on BP. Although many of these syndromes were known for decades, their molecular genetic basis has only recently been elucidated. Many of the genes identified involve either renal electrolyte transport molecules or adrenal steroid metabolic pathways controlling electrolyte balance (141, 272).

The question arises as to whether the same loci involved in monogenic variation in BP in humans are responsible for quantitative variation in BP through more subtle genetic variants. For the loci of the epithelial sodium channel involved in Liddle’s syndrome, the answer appears to be no in humans (18, 181, 182) and rats (69, 88, 123). In contrast, it could be argued that the allelic variation in 11β-hydroxylase in Dahl rats does provide a positive example of subtle quantitative variation in a steroid biosynthetic pathway causing an increment of BP (23, 26, 162, 190–193), but the point has not been proven by precise gene substitution experiments.

The possible Mendelian segregation of BP was observed by Tanase (250) in a cross of spontaneously hypertensive rats (SHR) and Donryu rats and would seem to have been confirmed in a four-way cross by the same author (251). Such a phenomenon has not been observed in extensive work by others using SHR, and such a powerful genetic locus in rats has not been mapped. Its existence remains problematic.

II. ANIMAL MODELS OF HYPERTENSION

Because human essential hypertension is a complex disease, researchers have selectively bred rats for high BP to provide animal models for the disease. In some cases, animals were also selected for low BP and/or a random-bred unselected stock from which the hypertensive strain was developed, was also maintained. These models and their initial references are listed in Table 1. Brief reviews on development of most of the rat strains and the mouse strains are available (65, 152). Because these strains are critical to any genetic analysis, brief comments on their genetic status and availability are given below.

The genetically hypertensive (GH) rats are a fully inbred strain (20 or more generations of brother × sister matings). Although they are not widely available, a colony is maintained by Eugene Harris at the Department of Surgery, University of Otago (PO Box 913, Dunedin, New Zealand; e-mail: jean.harris@stonebow.otago.ac.nz). The rats were selectively bred on the basis of BP without any dietary or environmental provocative stimuli.
Dahl salt-sensitive (S) and Dahl salt-resistant (R) rats were bred on the basis of their BP after being fed a high-salt (8% NaCl) diet. There is a strong interaction on BP between strains and dietary NaCl. The original selected rats were maintained as outbred stocks but were continually selected for divergence of BP on a high-salt diet (93). These outbred stocks are commercially available (Harlan Sprague-Dawley, PO Box 29176, Indianapolis, IN; www.harlan.com) and are designated DS (Brookhaven) for Dahl salt sensitive and DR (Brookhaven) for Dahl salt resistant. The outbred stocks are of little value because inbred strains are available. Inbred strains were developed from Dahl’s outbred (Brookhaven) stocks (194) and are designated SS/Jr for the Dahl salt sensitive (Rapp) and SR/Jr for Dahl salt resistant (Rapp). The definitive colonies of these strains are maintained by myself (John Rapp, Dept. of Physiology and Molecular Medicine, Medical College of Ohio, 3035 Arlington Avenue, Toledo, OH 43614-5804; e-mail: jrapp@mco.edu). The SS/Jr and SR/Jr strains are commercially available (Harlan Sprague-Dawley, see above; and Mollegaard Breeding and Research Centre, DK-4623 Li, Skensved, Denmark; e-mail: molgene@inet.uni-c.dk). Another set of inbred strains was developed from Dahl’s outbred stocks at Brookhaven National Laboratory (Upton, NY) by Iwai and Heine (94). These strains have been designated Dahl-Iwai S and Dahl-Iwai R and are apparently only available in Japan (Tsukuba Research Laboratories, Aisai, 5–1-3 Tokodai, Tsukuba, Ibaraki 300–26, Japan) (280).

Unfortunately, the inbred SS/Jr rats commercially available from Harlan Sprague-Dawley were genetically contaminated in 1992–1993 (140, 209). A test of recent commercial stocks indicated that this problem was apparently corrected (264).

The SHR was selectively bred for high BP without any provocative dietary or environmental stimuli by Okamoto and Aoki (175) in Kyoto, Japan. The SHR and “control” stock from Wistar-Kyoto rats (WKY) were imported by the National Institutes of Health (NIH) in the United States before either strain was fully inbred and distributed to commercial suppliers. This created the undesirable situation of genetic differences among various colonies of SHR and WKY from commercial suppliers in the United States (130, 131, 165, 214) and between the colonies in the United States and Japan (169).

Before full inbreeding, a substrain of SHR with exceptionally high BP was found to be more susceptible to stroke than other SHR substrains. This substrain was subsequently selectively bred for a high incidence of stroke (176, 281, 282); the stroke-prone SHR are designated SHRSP. Although exceptionally high BP is clearly a

### Table 1. Rodent strains selectively bred for blood pressure

<table>
<thead>
<tr>
<th>Strain</th>
<th>Lines</th>
<th>Original Stock</th>
<th>Year First Reported (Reference No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Zealand (Dunedin): genetically hypertensive (GH) rats</td>
<td>H, C</td>
<td>Wistar derived</td>
<td>Smirk and Hall, 1958 (234)</td>
</tr>
<tr>
<td>USA (Brookhaven): Dahl salt-sensitive (S) and Dahl salt-resistant (R) rats</td>
<td>H, L</td>
<td>Sprague-Dawley</td>
<td>Dahl et al., 1962 (30, 31)</td>
</tr>
<tr>
<td>Japan (Kyoto): spontaneously hypertensive rats (SHR)</td>
<td>H</td>
<td>Wistar derived</td>
<td>Okamoto and Aoki, 1963 (175)</td>
</tr>
<tr>
<td>Japan (Kyoto): spontaneously hypertensive rats-stroke prone (SHRSP)</td>
<td>H</td>
<td>Wistar derived</td>
<td>Okamoto et al., 1974 (176)</td>
</tr>
<tr>
<td>Israel (Jerusalem): DOCA salt-sensitive (SBH) and resistant (SBN) rats</td>
<td>H, L</td>
<td>Unknown</td>
<td>Ben-Ishay et al., 1972 (7)</td>
</tr>
<tr>
<td>France: Lyon hypertensive (LH), Lyon normotensive (LN), and Lyon low blood pressure (LL) rats</td>
<td>H, C, L</td>
<td>Sprague-Dawley</td>
<td>Dupont et al., 1973 (53)</td>
</tr>
<tr>
<td>Italy: Milan hypertensive strain (MHS) and Milan normotensive (MNS) rats</td>
<td>H, C</td>
<td>Wistar</td>
<td>Bianchi et al., 1974 (8)</td>
</tr>
<tr>
<td>The Netherlands (Utrecht): fawn-hooded hypertensive (FH1) and fawn-hooded low blood pressure (FLH) rats</td>
<td>H, L</td>
<td>Greman brown × white Lashley</td>
<td>Kuijpers and Grusy, 1984 (124)</td>
</tr>
<tr>
<td>Russia (Novosibirsk): inherited stress-induced arterial hypertension (ISIAH) rats</td>
<td>H</td>
<td>Wistar derived</td>
<td>Markel, 1985 (158)</td>
</tr>
<tr>
<td>Czech Republic: Prague hypertensive rat (PHR), Prague normotensive rat (PNR)</td>
<td>H, L</td>
<td>Wistar derived</td>
<td>Heller et al., 1993 (80)</td>
</tr>
</tbody>
</table>

**Mouse**

|       |       | Eight-way cross of inbred strains | Schlager, 1974 (217) |

**Hamster**

|       |       | Cardiomyopathic × gold Syrian hamsters | Thomas et al., 1997 (252) |

Kinds of lines developed in each model were as follows: H, line selected for high blood pressure; C, control line, unselected, random bred; L, line selected for low blood pressure.
factor in stroke of SHRSP, genetic factors in addition to those operating through BP also are likely to be involved. This was shown by producing $F_2$ and both backcross populations from SHR and SHRSP. Thus the three populations are $F_2$(SHRSP × SHR) × SHR, $F_2$(SHRSP × SHR), and $F_2$(SHR × SHR) × SHR, and they have 25, 50, and 75% SHRSP genes, respectively. All the populations were fed a high salt intake to remove BP differences among populations (all BP became very high), but there was still a positive relationship between the average percentage of SHRSP genes and the incidence of cerebrovascular lesions across populations (170).

The SHR and WKY are widely commercially available in the United States (e.g., Harlan Sprague-Dawley, see above; Charles River Laboratories, 251 Ballardvale St., Wilmington, MA 01887; www.crl.com; Taconic, 273 Hanover Avenue, Germantown, NH 12526; www.taconic.com) or in Europe (Møllegaard Breeding and Research Centre, see above). The SHRSP are not commercially available, but colonies are maintained around the world (Genetic Resource Section, NIH, Building 14F, Room 101, Bethesda, MD 20892; www.nih.gov/od/orr/dirs/vrp/nihagr.htm; Dr. Y. Yamori, Graduate School of Human and Environmental Studies, Kyoto University, Yoshida Nihonmatsu-cho, Sakyo-ku, Kyoto 606–8501, Japan; Fax: 81 75 7532997; Dr. R. C. Webb, Dept. of Physiology, Univ. of Michigan, 7812 Medical Science Building II, Ann Arbor, MI 48109–0622; Fax: 734 936 8813; Dr. D. Ganten, Max-Delbrück-Centrum für Molekulare Medizin, Robert-Rössle-Strasse 10, D-13125 Berlin-Buch, Germany, Fax: 49 30 949 7008; Dr. A. F. Dominiczak, Dept. of Medicine and Therapeutics, Gardiner Institute, Western Infirmary, Glasgow G11 7T, UK; Fax: 44 141 211 1763; e-mail: ad7e@clinmed.gla.ac.uk). Genetically defined SHRSP/Izm, SHR/Izm, and WKY/Izm are available from the Disease Model Cooperative Research Association (86–2 Jodojishimobabacho, Sakyo-ku, Kyoto 606–8413, Japan; Fax: 81 75 761 2382).

The SHR are hyperactive in an open-field environment compared with WKY. Blood pressure and hyperactivity were not correlated in an $F_2$ population derived from SHR and WKY, implying independent genetic determinants (81). To confirm that there was no obligate genetic association between hyperactivity and hypertension, two strains with contrasting properties were selectively bred from an $F_2$ population derived from a cross of SHR and WKY. One strain was selected for hyperactivity and the other for high BP. In fact, a hypertensive nonhyperactive strain (WKHT) and a normotensive hyperactive (WKHA) strain were successfully developed (48, 82). These strains have been inbred for at least 25 generations of brother-sister mating and are maintained by Dr. C. F. Deschepper (Institut de Recherches Cliniques de Montreal, 110 Pine Ave. West, Montreal, Quebec, Canada H2W 1R7; Fax: 514 987 5585; e-mail: deschech@ircm.qc.ca).

Sabra hypertension-prone (SBH) and Sabra hypertension-resistant (SBN) rats were bred on the basis of the BP response to unilateral nephrectomy, treatment with deoxycorticosterone acetate, and 1% NaCl to drink (7). The SBH and SBN colonies have recently been rederived from Ben-Ishay’s stock and are currently fully inbred (276). The colony has been moved from Hebrew University in Jerusalem to Ashkelon, Israel (Dr. Y. Yagil, Laboratory of Molecular Medicine, Ben-Gurion University, Barzilai Medical Center Campus, Ashkelon 78306, Israel; Fax: 972 7 674 5824; e-mail: labmomed@bgumail.bgu.ac.il).

The Lyon strains of rats were selected for high (LH) or low (LL) BP, and a third line (LN) was selected for normal BP without special dietary or environmental challenges (53). At last report, the BP of the LL and LN rats were similar (215), and both of course were lower than LH rats. The strains are presumably inbred, but a report in 1993 found a few alleles at genetic markers still to be segregating (49). The strains are only available in France (Dr. J. Sassard, Département de Physiologie et Pharmacologie Clinique, CNRS ESA 5014, Faculté de Pharmacie, 8 Avenue Rockefeller, 69373 Lyon Cedex 08, France; Fax: 33 478 777118).

The Lyon strains were selected for high (MHS) and low (MNS) BP without special dietary or environmental challenges (8). The strains are fully inbred and are maintained in the United States (Genetic Resource Section, NIH, see above) or in Italy (G. Bianchi, Div. of Nephrology, Universit di Milano, Ospedale San Raffaele, Via Olgettina 60, 1–20132 Milan, Italy; Fax: 39 02 2643 2384; Patrizia Ferrari, PRASSIS Research Institute, Via Forlanini 3, 20199 Settimo Milanese, Milan, Italy; Fax: 39 02 3350 0408).

The fawn-hooded (FH) rat was obtained initially from a cross of German-brown and white Lashley rats. It is characterized by a platelet abnormality resulting in a mild bleeding disorder (255) and by glomerular sclerosis (118). A colony was established at Unilever Research Laboratories, Vlaardingen, The Netherlands, which was maintained as a closed outbred colony until the mid 1980s, at which point it was recognized that the rats had spontaneous hypertension and renal lesions resulting in proteinuria (124–126). Selective inbreeding was performed for high BP (FHH strain) and for normotension (FHL strain) (188, 258). The FHH strain is homozygous recessive for three coat color genes: red-eyed dilution ($r$), nonagouti ($a$), and hooded ($h$) (14). The FHH and FHL strains are maintained by A. P. Provoost (Dept. of Pediatric Surgery, Erasmus University Rotterdam, The Netherlands; e-mail: provoost@heel.fgg.eur.nl).
MHS strains) that were also done on conscious restrained rats. The ISIAD rats are maintained by A. L. Markel (Institute of Cytology and Genetics, Russian Academy of Science, Siberian Branch, 630090, Novosibirsk 90, Russia; e-mail: markel@cgi.nsk.su).

Rats have been selectively inbred for high or normal BP in Prague producing the Prague hypertensive rat (PHR) and the Prague normotensive rat (PNR) (80). Both strains originated from the same single pair of breeders. The strains are maintained by J. Heller (Institute of Clinical and Experimental Medicine, Vídensk 800, CS-146 22, Prague 4, Czech Republic).

Mice have also been selectively bred for BP. In the case of the mice, the selection experiment was more rational than much of the rat work. With some of the rat breeding, the strains were started from a narrow genetic base (sometimes one pair of rats) that obviously limits the alleles entering the experiment, and selection and inbreeding were practiced concomitantly. The latter procedure is not optimal for separating contrasting alleles into the divergent strains. The mouse selection experiment started from an 8-way cross of inbred strains, and selection was applied for 23 generations before the lines were inbred by at least 40 generations of brother-sister mating as of 1997. Selection was done for both high and low BP, and a random-bred line was also maintained and also subsequently inbred (217–219). The mouse strains are designated BPH/2, BPL/1, and BPN/3 for the high, low, and normotensive strains, respectfully, and are available from the Jackson Laboratory (Animal Resources, 600 Main Street, Bar Harbor, ME 04609–1500; www.jax.org).

Cursory review of Table 1 indicates that there is not much genetic diversity in the origin of hypertensive rat strains; most were developed from Wistar-related stocks. Two strains were developed from Sprague-Dawley stock, but Sprague-Dawley and Wistar have a common origin (146). The fawn-hooded rats apparently have a more distant origin and thus may be of unique value. The commonality of the hypertensive strains can be partially circumvented, however, by crossing a hypertensive strain to another origin and thus may be of unique value. The commonality of the hypertensive strains can be partially circumvented, however, by crossing a hypertensive strain to another origin and thus may be of unique value. The commonality of the hypertensive strains can be partially circumvented, however, by crossing a hypertensive strain to another origin and thus may be of unique value. The commonality of the hypertensive strains can be partially circumvented, however, by crossing a hypertensive strain to another origin and thus may be of unique value. The commonality of the hypertensive strains can be partially circumvented, however, by crossing a hypertensive strain to another origin and thus may be of unique value. The commonality of the hypertensive strains can be partially circumvented, however, by crossing a hypertensive strain to another origin and thus may be of unique value. The commonality of the hypertensive strains can be partially circumvented, however, by crossing a hypertensive strain to another origin and thus may be of unique value.

III. GENOMIC RESOURCES

Most of the resources required in a molecular genetic analysis of QTL are available for the rat. The most useful genetic markers are short tandem repeated sequences of DNA consisting of mono-, di-, tri-, or tetranucleotide repeated sequences, or so-called microsatellites, also referred to as short tandem repeats (STR) or simple sequence length polymorphism (SSLP) markers, or simple sequence repeats (SSR). These repeated sequences are very numerous and are scattered throughout the noncoding DNA of eukaryotes. They are also highly polymorphic with regard to length and are easily genotyped using the polymerase chain reaction (PCR) (4, 239, 268, 269). Initially, the oligonucleotide primers for the PCR amplification and genotyping of rat microsatellites were obtained from sequences that were in databases as a consequence of sequencing known genes or limited screening for microsatellites (67, 129, 203, 225, 288). Subsequently, several laboratories developed larger numbers of markers by screening small-insert genomic libraries for microsatellites and prepared genetic linkage maps of all the rat chromosomes using such markers (10, 13, 29, 99, 163, 184, 263) (www.genome.wi.mit.edu; www.well.ox.ac.uk/pub/genetics/ratmap). Such linkage maps with readily available markers are certainly the single most important genomic resource for genetic analysis of any quantitative trait. Polymorphism data for microsatellites on 48 strains of rats is available at www.informatics.jax.org/rat/.

Rat chromosomes have been flow sorted by Hoee et al. (87) and Shepel and co-workers (228, 229). Chromosome-specific libraries were used to develop microsatellite markers for chromosomes (chr) 1 (70), chr 2 (43, 227), chr 3 (38), chr 5 (42), and chr 10 (51, 52). Because flow sorting did not separate certain chromosomes, such libraries also sometimes yielded markers on multiple chromosomes (47, 228, 244). There are two reports of chromosome dissection on chr 10 to obtain genetic markers (6, 112).

Genome resources are more highly developed in the mouse than in the rat. It has been possible therefore to utilize mouse microsatellite PCR primer pairs in the rat. About 20% of such mouse markers amplify a specific product from rat DNA, but only ~4% of the mouse markers provided polymorphic markers (46, 115).

Somatic hybrid cell panels are a useful resource for localizing genes onto chromosomes. In this technique, rat and mouse cultured somatic cells are fused. For unknown reasons, one or more rat chromosomes will be stably retained at random in some of the mouse cells. A panel of such cell lines known by cytologic techniques to retain one or more rat chromosomes can be tested for the presence of specific rat genes or markers to establish linkage to rat chromosomes. Two rat/mouse hybrid-cell panels are available for the rat, one developed in Belgium by Szpirer and co-workers (245–247) and one developed in Japan at Kyoto University (285, 286).

An additional potentially important technique for preparing genetic maps utilizes radiation hybrid-cell (RH) panels (137, 265). In this technique, a donor (e.g., rat) cultured somatic cell line is lethally irradiated with X-rays and then fused to somatic cell lines of another species
(e.g., hamster). Rat chromosome fragments will be integrated into the hamster chromosomes, and a series of relatively stable RH cell lines is established. The closer two rat loci are to each other on a rat chromosome, the more likely they will be retained in the same RH cell line. If the rat loci are far apart, or on different chromosomes, they will be much less likely to be retained in the same RH cell lines. Data on the presence or absence of a rat gene/marker usually detected by PCR in the RH panel can be used to construct genetic maps. First-generation RH maps are available for the mouse (62, 166). A rat/hamster RH panel developed by Peter Goodfellow's laboratory at Cambridge University in the United Kingdom is currently available from Research Genetics (Huntsville, AL; www.resgen.com) or the Resource Centre of the German Human Genome Project (www.rzpd.de). Extensive radiation hybrid cell maps of rat chromosomes containing 5,255 markers have recently been published (267) (www.well.ox.ac.uk/rat_mapping_resources). An additional extensive set of radiation hybrid cell and linkage maps for the rat are also available (242) (http://goliath.ifrc.mcw.edu/LGR/research/rhp/index.html; http://curatools.curagen.com/ratmap).

Once a gene of interest is very well localized on a genetic map, a cloning project for that gene will almost certainly require the use of large-insert DNA libraries for the rat prepared using a yeast artificial chromosome (YAC) and/or a P1 bacteriophage vector, the so-called P1-derived artificial chromosome (PAC) (http://bacpac.med.buffalo.edu/rat_pac.html). These important reagents have recently become available for the rat (16, 71, 273), and an anchored YAC framework map is available (15).

The rat linkage maps have been oriented with the cytogenetic maps by placing clones of known genes, cosmid, or YAC clones containing known microsatellite markers on the chromosomes by fluorescent in situ hybridization (FISH) (3, 248). Also, comparisons of the chromosomal maps for mouse, human, and rat are available (138, 279). See also www.otsuka.genome.ad.jp/ratmap for comparative maps. Synteny means that two loci are in the same linkage group; conserved synteny means that two linked loci in one species have homologous loci that are also linked in another species. Often, the order of the linked loci is also conserved between species. This information is very helpful in locating candidate loci in QTL regions or in developing genetic markers in specific regions of rat chromosomes by knowing what loci are in the homologous target region of human or mouse.

A recent new technology is the ability to construct microarrays of thousands of different DNA molecules for the purpose of hybridization to labeled DNA. The technology and its use have recently been reviewed (293). The technique is useful, for example, to array sequences from known genes to detect the relative abundance of mRNA molecules in samples (11, 50). Again, such reagents for the human and mouse genome are more available than for the rat, but a recent article utilized a microarray of 10,000 cDNA clones, from a normalized library of rat heart (1).

IV. STRATEGIES FOR DEFINING QUANTITATIVE TRAIT LOCI FOR BLOOD PRESSURE

Given strains of rats with markedly different BP, it is obvious that there must be a genetic basis for these differences that are passed from one generation to the next. It follows logically that genetic techniques must be employed to define and understand these genetic differences. This has only become a practical possibility with the advent of large numbers of genetic markers throughout the genome.

A. Linkage Analysis in Segregating Populations

In the case of a single Mendelian trait, the genotype of an individual at the locus causing the variation in the trait can be inferred from observing the phenotype. This is not so in the case of a quantitative trait such as BP where alleles at multiple genetic loci influence the trait. Thus, knowing the phenotype, i.e., BP, yields no unique information about the genotype at any given single locus, because BP is the net effect across many loci.

For example, consider the four individuals from a segregating population with genotypes shown below. Let A, B, C, D, and E be loci on five different chromosomes that influence BP, i.e., so-called BP QTL. Assume there are two alleles at each locus such that an allele with subscript 1 (A1, B1, C1, etc.) lowers BP by 5 mmHg (minus allele) and an allele with subscript 2 (A2, B2, C2, etc.) increases BP by 5 mmHg (plus allele) around an overall mean of 150 mmHg. Assume as a first approximation that the effects at all loci are additive. For example, individual 1 has eight subscripts 1 (8 x 5 = 40 mmHg) and two subscripts 2 (2 x +5 = +10 mmHg), so the net effect of the plus and minus effects is 40 + 10 = 50 mmHg about an overall mean of 150 mmHg yields 120 mmHg for individual 1. The BP for the other individuals are calculated similarly

<table>
<thead>
<tr>
<th>Individual</th>
<th>Genotype</th>
<th>Blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A1A1B1B1C1C1D1D1E1E1</td>
<td>120 mmHg</td>
</tr>
<tr>
<td>2</td>
<td>A1A2B1B1C1C1D1D1E1E1</td>
<td>120 mmHg</td>
</tr>
<tr>
<td>3</td>
<td>A1A2B2B2C2C2D2D2E2E2</td>
<td>150 mmHg</td>
</tr>
<tr>
<td>4</td>
<td>A1A1B2B2C2C2D2D2E2E2</td>
<td>180 mmHg</td>
</tr>
</tbody>
</table>

Obviously knowing that individuals 1 and 2 have the same BP does not define the genotype at, for example, QTL A which is A1A1 in individual 1 and A2A2 in individual...
2. Similarly, individuals 1 and 4 have markedly different BP but have the same genotype, A1A1, at QTL A.

Unambiguous genotyping at a genetic locus is required to place the locus on genetic maps. For simple Mendelian traits, the genotype can be inferred from the phenotype, and the locus involved can be placed on the genetic map as the first step in cloning and identifying a previously unknown locus causing the Mendelian trait. This is the usual strategy for cloning of genes causing genetic diseases inherited in a simple Mendelian fashion. Although such accurate map localization is impossible for (essentially all) BP QTL by studying BP per se, DNA-based genetic microsatellite markers are inherited as simple Mendelian traits and are readily placed on genetic linkage maps as noted in section III. It is logical then to determine which genetic markers cosegregate with BP to find the approximate location of the QTL.

It is worth understanding how this cosegregation works in the context of experimental mammalian genetics. Consider a marker locus M, which is a microsatellite marker locus on the same chromosome and closely linked to BP QTL A. Suppose the alleles M1 and M2 at locus M and alleles A1 and A2 at QTL A are organized as follows in two inbred parental strains P1 and P2. Let A1 be a minus allele that lowers BP and A2 be a plus allele that increases BP. In the diagram above, each line represents one chromosome of a pair of chromosomes. Thus marker allele M1 is linked to the QTL A minus allele A1, and marker allele M2 is linked to the QTL A plus allele A2. Because P1 and P2 are inbred strains, both the marker and QTL loci are homozygous for their respective alleles.

P1 is crossed to P2 and the F1 rats are intercrossed to produce a large F2 population.

\[
P_1 \times P_2 \quad (M_1M_1) \downarrow (M_2M_2) \quad F_1 \quad (M_1M_2)
\]

\[
F_1 \times F_1 \quad (M_1M_2) \downarrow (M_1M_2) \quad F_2 \quad (1M_1M_1;2M_1M_2;1M_2M_2)
\]

The F2 rats are phenotyped for BP and genotyped at the microsatellite marker locus by PCR. The marker alleles will segregate in Mendelian fashion 1M1M1;2M1M2;1M2M2. The BP of the rats in the three marker classes can be compared by a one-way ANOVA. In the example constructed above, there will be BP differences among the marker classes because the marker is linked to a BP QTL. In the extreme case where locus M is very closely linked to QTL A, the marker class M1M1 will consist of rats with genotype A1A1 at the QTL, M2M2 will represent rats with QTL genotype A2A2, and M1M2 will represent rats with QTL genotype A1A2. Because alleles at QTL on other chromosomes (loci B, C, D, and E in the example above) will assort at random with respect to M (and A), there will be no consistent enrichment of the alleles on other chromosomes in one marker classes at M compared with another marker class at M, and the plus and minus BP effects at the QTL other than at locus A will cancel each other out. The segregation of alleles at the other QTL will, however, increase the BP variance within a marker class at M, thus making the detection of BP effects associated with M more difficult statistically. Examples of single marker linkage to BP are given in Table 2; the marker on chr 2 is linked to BP, but the one on chr 3 is not. As presented above, the marker M was very close to the BP locus A. In general, this will not be the case. As the chromosomal map distance between M and A increases, the chances for recombination by chromosomal crossing over between M and A increases. This will degrade the accuracy with which the genotype at M predicts the genotype at A, and consequently, the BP cosegregating with M will decrease as the distance between M and A increases.

Figure 1 shows meiosis with or without recombination in an F1 heterozygote obtained by crossing the strains P1 and P2 described above. In Figure 1, top, where there is no recombination, M1 stays coupled with A1 and M2 with A2. In Figure 1, bottom, recombination occurs by chromosomal crossing over between loci M and A, and as a consequence, M1 is as likely to be coupled to A1 as it is to A2, and similarly M2 is as likely to be coupled to A1 as A2. More realistically, the meiotic products (and thus the population of rats produced from these gametes) will be a mixture of the events depicted in Figure 1, top and bottom. The closer M and A are to each other, the more likely the original parental coupling of M1 to A1 and M2 to

<table>
<thead>
<tr>
<th>Marker Locus</th>
<th>Rat Chromosome</th>
<th>Blood Pressure by Marker Genotype</th>
<th>P Value (1-way ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naka1</td>
<td>2</td>
<td><strong>166 ± 3.3</strong></td>
<td><strong>173 ± 2.4</strong></td>
</tr>
<tr>
<td></td>
<td><strong>(n = 49)</strong></td>
<td><strong>(n = 74)</strong></td>
<td><strong>(n = 36)</strong></td>
</tr>
<tr>
<td>Edn3</td>
<td>3</td>
<td><strong>174 ± 3.4</strong></td>
<td><strong>176 ± 3.2</strong></td>
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<tr>
<td></td>
<td><strong>(n = 42)</strong></td>
<td><strong>(n = 75)</strong></td>
<td><strong>(n = 42)</strong></td>
</tr>
</tbody>
</table>

Values are means ± SE for systolic blood pressure; n, no. of rats in each genotypic class. W, allele from WKY rats; S, allele from Dahl salt-sensitive rats. [Data from Deng and co-workers (39, 40).]
A2 is to be retained in a high percentage of gametes, and the further the distance the less likely this coupling is preserved.

If BP linkage to a single marker has been found, the problem arises: Where is the QTL in relation to the marker and how large is the effect of the QTL on BP? With reference to the data in Table 2, the marker at Nakal1 on chr 2 is associated with a BP effect of 21 mmHg (the difference between BP of rats with SS genotype and those with WW genotype at Nakal1). This could arise from a QTL very closely linked (or identical) to Nakal1 with an effect of 21 mmHg, or it could arise from a QTL of larger effect at a further distance from the Nakal1 locus. Without precise information on the location of an unknown locus on a chromosomal map, it is impossible to identify it.

It is, of course, possible to study loci along a chromosome for linkage to BP considering each locus separately. Representative data are given in Figure 2. The BP effect is at a maximum around the Ae3 locus (anion exchanger 3) and drops off on either side of Ae3 as the linkage map distance from Ae3 increases. Nevertheless, such data do not provide any formalized statistical estimate of QTL location and do not localize the QTL well enough by inspection to attempt to identify it by positional cloning.

The twofold problem of proving the existence of a QTL and of finding its map position has received much attention by statistical geneticists. There are good textbooks on the subject (148, 153), and several different techniques have been developed, all of which are mathematically intense and require computer implementation. Two popular and readily available programs are MAPMAKER/QTL for PC and other platforms (contact Eric Lander, e-mail: mapmaker@genome.wi.mit.edu) (133, 134, 143, 144, 180) and Map Manager QT for MacIntosh computers (contact Kenneth Manly, e-mail: kmanly@mcbio.med.buffalo.edu). Others are listed by Paterson (179) and have recently been reviewed (156).

One approach to QTL analysis utilizes maximum likelihood techniques and calculates a statistic, the LOD score (which is the log of the ratio of the likelihood of there being a QTL present vs. the likelihood of no QTL being present at a particular map position). The LOD scores are calculated at many selected points in an interval between markers and plotted versus map position. The QTL effect enters the likelihood equations through the observed BP data and the QTL map position enters the equations through the probability of a given QTL genotype given the flanking marker genotypes for each individual and the assumed QTL position in the interval. Although the mathematical development of such methods is hardly appropriate here, it is worth presenting how to interpret such calculations and to understand their limitations.

Figure 3 gives an LOD plot for the same data given in Figure 2. The peak of the LOD plot gives the most likely location of the QTL, and the height of the peak is a measure of statistical significance. The height of the LOD peak in Figure 3 (LOD = 4.8) tests for the existence of a BP QTL. As a rough approximation, a LOD >3 has often been considered to be significant. A more precise evaluation of how to interpret LOD values, however, shows that factors such as the size of the genome, the underlying assumed genetic model for the QTL effects (i.e., additive, dominance/recessive, or unconstrained model), and the breeding paradigm (e.g., backcross or F2 population) all

---

**FIG. 1.** Gametes produced from meiosis without (top) and with (bottom) chromosomal crossing over (recombination). A single pair of chromosomes is shown which have replicated into 2 sister chromatids that are still joined at centromere during synapse. M is a marker locus with alleles M1 and M2. A is a quantitative trait locus (QTL) with alleles A1 and A2.
influence interpretation of the LOD plot. Lander and Kruglyak (135) have made calculations accounting for such factors and give threshold values for LOD scores under various conditions. The appropriate thresholds are shown in Figure 3, where the dotted line indicates the threshold for suggestive significance (LOD 5 1.9), and the solid line is the threshold for significance (LOD 5 3.3) under the conditions of the particular experiment in Figure 3. Because the peak value (LOD 5 4.8) in Figure 3 is greater than the threshold for significance, the data are interpreted to mean that a QTL for BP on chr 9 is segregating in the specific cross made.

The position of the peak in Figure 3 gives the most likely position for the QTL, but its exact location remains unknown. The support interval defined by one LOD unit around the peak gives an 60–95% probability of containing the QTL (155); simulation studies suggest that support intervals based on two LOD units should be used (256). Construction of these LOD support intervals is shown in Figure 3.

The length of the confidence interval will be a function of the size of the experimental population, the magnitude of the QTL effect, and marker density. It has been shown (35) that increasing marker density beyond one marker every 5–10 cM does not significantly improve the QTL localization. The larger the phenotypic effect of a QTL, the easier it will be to detect and localize it. Most BP QTL described so far have a modest effect individually accounting for only 5–15% of the total BP variance in experimental populations. Unfortunately, this means that with experimental populations of reasonable number (200–300 backcross or $F_2$ rats), BP QTL are likely to be localized only to about a 20- to 35-cM interval by linkage analysis (90, 256). In actual practice, this has been the

<table>
<thead>
<tr>
<th>CHROMOSOME 9</th>
<th>Blood Pressure by Genotype</th>
<th>One-way ANOVA</th>
<th>Blood Pressure Effect</th>
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<tr>
<td></td>
<td>SS</td>
<td>SR</td>
<td>RR</td>
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<tr>
<td>D9Rat1</td>
<td>183 (n=64)</td>
<td>177 (n=95)</td>
<td>170 (n=50)</td>
</tr>
<tr>
<td>D9Rat2</td>
<td>182 (n=61)</td>
<td>180 (n=105)</td>
<td>164 (n=40)</td>
</tr>
<tr>
<td></td>
<td>186 (n=71)</td>
<td>179 (n=114)</td>
<td>160 (n=47)</td>
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<td></td>
<td>181 (n=54)</td>
<td>179 (n=92)</td>
<td>160 (n=38)</td>
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<td></td>
<td>176 (n=61)</td>
<td>180 (n=97)</td>
<td>164 (n=55)</td>
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<td>181 (n=21)</td>
<td>177 (n=55)</td>
<td>176 (n=19)</td>
</tr>
<tr>
<td></td>
<td>179 (n=57)</td>
<td>179 (n=106)</td>
<td>171 (n=47)</td>
</tr>
</tbody>
</table>

![FIG. 2. Linkage analysis of genetic markers with blood pressure (BP) on chromosome (chr) 9 in an $F_2(S \times R)$ population. Genetic linkage map is shown at left. Rats are categorized by marker genotype at each marker. S, allele from Dahl salt-sensitive S rats; R, allele for Dahl salt-resistant R rats; n, number of rats; cM, centiMorgan. BP of 3 genotypic classes are compared by a 1-way ANOVA at each marker. [From Rapp et al. (198). Copyright 1998 Academic Press.](http://physrev.physiology.org/).]
A 20- to 35-cM interval is much too large to attempt positional cloning of the QTL, so other techniques are needed. As will be seen in section V B, construction of congenic strains and congenic substrains using the information obtained from linkage analysis has been a popular approach. Positional cloning means the identification of loci causing a phenotype by the use of linkage analysis and study of the DNA structure and function in the chromosomal interval defined by the linkage.

In addition to the inherent statistical imprecision with which a QTL can be localized, the possibility exists that two or more QTL may be linked on the same chromosome. This may, or may not, result in multiple peaks in the LOD plot, depending on how close the QTL are and the phase of the alleles. If two QTL are far enough apart to segregate independently (e.g., 80 cM apart), then two peaks can be observed (133). If two QTL are close and their alleles are in repulsion (see Fig. 4), the effects of the two QTL may cancel each other out, and neither may be observed. If the alleles of the two linked QTL are in coupling phase (see Fig. 4), then 1) a single LOD-plot peak or a broad plateau may be observed if the QTL are close, 2) two peaks may be observed if the QTL are far apart, or 3) a “ghost” peak between the two QTL may be observed.

**FIG. 3.** Logarithm of the odds favoring linkage of BP to genetic markers (LOD plot) is shown for the same data as in Figure 2. Dotted line at LOD = 1.9 is threshold for suggestive statistical significance, and solid line at LOD = 3.3 is threshold for significant linkage to BP. Construction of 1-LOD and 2-LOD support intervals to right of linkage map are shown. A congenic strain constructed by introgressing the Dahl R rat region into the Dahl S rat is shown by larger solid bar to left of linkage map; open regions at ends of the congenic bar are intervals in which recombination occurred. The congenic strain had a BP 19 mmHg lower (P < 0.0001) than S rats, implying that a minus BP allele was successfully moved from R to S strain. [From Rapp et al. (198). Copyright 1998 Academic Press.]
if the QTL are ~40 cM apart and the markers are widely spaced (~20 cM) (72, 161, 274). An example of a LOD plot with multiple peaks is shown in Figure 5. Obviously, this is difficult to interpret with regard to the number of linked QTL and their location.

It needs to be emphasized that a given linkage result arising from a segregating population obtained from crossing two inbred strains is often unique to these two strains. This is because crosses of other pairs of strains may not be functionally polymorphic at a given QTL. Functionally polymorphic means that at the locus accounting for a QTL there is an allelic DNA difference that causes functional differences in expression or activity of the locus-encoded product. Obviously, if a pair of inbred strains are homozygous for the same allele at a QTL, there will be no effect of this QTL on the quantitative trait in segregating populations derived from these strains. Other considerations causing QTL results to be cross-specific include the genetic background that can influence the expression of a given QTL effect and that is unique to each pair of parental strains used to form the segregating population. Often the experimental conditions (diet, age of rats, etc.) vary between experiments, which may alter expression of a given QTL.

### B. Recombinant Inbred Strains

Recombinant inbred (RI) strains are produced by 1) crossing two inbred strains, 2) producing an F2 population from the F1 heterozygotes, and 3) selecting breeding pairs at random from the F2 population, each pair to be founders for an inbred strain produced by 20 generations of brother-sister matings. The process is illustrated in Figure 6. Recombination of the parental chromosomes occurs at meiosis in the F1 animals and in subsequent generations, but at the same time recombinant chromosomal regions are becoming fixed (homozygous) by brother-sister inbreeding. The resulting inbred strains are each a mosaic of the genetic material from the founding parental strains as illustrated in Figure 6, bottom, for a hypothetical chromosome. If a large enough panel of RI strains is produced, it can be used to produce genetic linkage maps and for detecting and locating QTL.

Linkage information from RI strains is fundamentally similar to that in a segregating population as illustrated in Figure 6, bottom. If two loci (M1 and M2) are close together on the same chromosome, they are likely to have fixed the same parental allele because the probability of a chromosomal crossover in a small distance is low. Thus, in Figure 6, each of the four RI strains illustrated have alleles from the same parental strain at M1 and M2 (the strains are 100% concordant for M1 and M2). The proba-
bility that two loci on the same chromosome are concordant among RI strains decreases as the distance between the loci increases. In Figure 6, only 50% of the RI strains are concordant at loci M1 and M3 because the loci are far apart. Information on the concordance of a panel of RI strains at various loci can of course be used to construct genetic maps (231). It is emphasized that the larger the number of RI strains available, the more accurate the genetic linkage and that the four strains illustrated in Figure 6 are hardly sufficient. Most panels of RI strains consist of 20–30 strains.

Animals within an individual RI strain are genetically identical. One of the advantages of RI strains is that the phenotype of each strain, for example, BP, can be carefully and repeatedly studied on many individuals from each strain. The RI strains can then be used for QTL analysis. In its simplest form, the genotype of each RI strain at a marker locus is determined, and the phenotypic values of the two groups are compared by a t-test; an individual strain at any locus is either homozygous for one parental allele or the other. Following the example in Figure 6, the strains that were BB or SS at the marker of interest would have their BP compared. If the two groups of strains had significantly different BP, this would be evidence for a QTL in the vicinity of the marker used.

Pravenec et al. (185) have developed a panel of 31 RI strains derived from spontaneously hypertensive rats (SHR/Ola) and a strain of Brown-Norway (BN.lx/Cuba) carrying genes for polydactyly. This panel of RI strains is maintained in two places: Michael Pravenec, Institute of Physiology, Czech Academy of Sciences, Vídeonsk 1083, CS-14220, Prague 4, Czech Republic; Fax: 420 2 475 2297; e-mail: pravenec@biomed.cas.cz; and Morton Printz, Department of Pharmacology-0636, 9500 Gillman Drive, Uni-
compatibility complex (RT1) on chr 20 (see sect. V). The HSP70 is located in the major histocompatibility complex (MHC) because they are linked closely on chr 20. (Some RFLP data are missing because some RI strains were not available for RFLP analysis.) Note also that most of strains carrying RFLP 4.4-kb fragment from SHR fall to right (higher BP) of diagram. Average of mean BP of BN fall to left (lower BP) and most of strains carrying 3.0-kb fragment of BP. Hamet et al. (74) showed that for a restriction fragment length polymorphism (RFLP) in 70-kDa heat shock protein (HSP70) locus is indicated diagrammatically below BP bar for each strain as either a 4.4- or a 3.0-kb fragment. Rats were also typed immunologically for allele carried by each strain at 70-kDa heat shock protein (HSP70) locus strains are arranged in order of increasing mean arterial pressure. RFLP polymorphism (RFLP) in RI strains obtained from SHR and BN rats. RI strains carrying SHR allele (143 mmHg) vs. 128 mmHg, 3 mmHg, 6 mmHg, and 9 mmHg, 12 mmHg, and 15 mmHg, respectively. Note 100% concordance between HSP70 RFLP fragment and RT1 haplotype because they are linked closely on chr 20. (Some RFLP data are missing because some RI strains were not available for RFLP analysis.) Note also that most of strains carrying RFLP 4.4-kb fragment from BN fall to left (lower BP) and most of strains carrying 3.0-kb fragment from SHR fall to right (higher BP) of diagram. Average of mean BP of strains carrying BN allele was lower (128 ± 3 mmHg) than BP strains carrying SHR allele (143 ± 6 mmHg). [Redrawn from Hamet et al. (74).]

![Diagram](https://example.com/diagram.png)

**FIG. 7.** Association of BP and a BamHI restriction fragment length polymorphism (RFLP) in RI strains obtained from SHR and BN rats. RI strains are arranged in order of increasing mean arterial pressure. RFLP allele carried by each strain at 70-kDa heat shock protein (HSP70) locus is indicated diagrammatically below BP bar for each strain as either a 4.4- or a 3.0-kb fragment. Rats were also typed immunologically for allele carried by each strain at 70-kDa heat shock protein (HSP70) locus strains are arranged in order of increasing mean arterial pressure. RFLP polymorphism (RFLP) in RI strains obtained from SHR and BN rats. RI strains carrying SHR allele (143 mmHg) vs. 128 mmHg, 3 mmHg, 6 mmHg, and 9 mmHg, 12 mmHg, and 15 mmHg, respectively. Note 100% concordance between HSP70 RFLP fragment and RT1 haplotype because they are linked closely on chr 20. (Some RFLP data are missing because some RI strains were not available for RFLP analysis.) Note also that most of strains carrying RFLP 4.4-kb fragment from BN fall to left (lower BP) and most of strains carrying 3.0-kb fragment from SHR fall to right (higher BP) of diagram. Average of mean BP of strains carrying BN allele was lower (128 ± 3 mmHg) than BP strains carrying SHR allele (143 ± 6 mmHg). [Redrawn from Hamet et al. (74).]

Regardless of the experimental design or the method of analysis, linkage data yield only a chromosomal region containing a gene (or genes) influencing BP that is too large for positional cloning. In addition to that, the statistical support for the existence of the BP QTL is often problematic. The limiting factors in linkage analysis are the modest phenotypic effects of most BP QTL and the limited number of chromosomal recombinant events that can be observed in segregating populations of practical size. One way around this impasse is the construction of congenic strains and then congenic substrains (136, 197).

The construction of a congenic strain is a standard procedure of experimental mammalian genetics (231) originating from the work of Snell (238) on histocompatibility loci. Figure 8 outlines the procedure for moving a polymorphic marker gene from one inbred (donor) strain to another inbred (recipient) strain. In Figure 8, the donor at marker locus M has genotype M1M1, and the recipient is M2M2. The two strains are crossed, and the F1 heterozygotes, M1M2, are backcrossed to the recipient strain. The backcross offspring will segregate 1M1M1:1M2M2. Heterozygote M1M2 are selected and backcrossed again to the recipient strain. This procedure is repeated for at least eight cycles of backcrossing at which point two heterozygotes are intercrossed to yield offspring in the ratio of 1M1M1:2M1M2:1M2M2. Two offspring homozygous M1M1 are bred to fix the M1 allele on the recipient background.

At each backcross, 50% of the loci outside of the region linked to the M locus that are segregating will become fixed for the recipient allele by chance. Thus the genetic background becomes progressively enriched for the recipient strain genes until the background is >99% recipient genes after the eighth backcross. Of course, donor genes linked to M1 will be “dragged” along with the M1 allele in this procedure. The congenic strain that results from selecting only one locus M will have flanking donor chromosomal segments on average equal to 100/N cM on each side (231), where N is the number of backcrosses. After eight backcrosses, the flanking DNA is on average 12.5 cM on each side of the selected marker, or 25 cM in size.

If M is a marker near a QTL, the above procedure may or may not move the donor QTL allele to the recipient strain depending on whether or not a recombination event occurred between M1 and the donor QTL allele.
during any of the backcrosses. To largely ensure that a linked QTL allele is moved to the recipient strain, one can easily select for donor alleles at markers along the chromosomal region of interest, always rejecting individuals with recombination in the target region and selecting for individuals that are heterozygous for all markers in the region. Thus the strategy is to select a large region in the construction of the initial congenic strain to ensure moving the QTL allele, the position of which is known only as a statistical approximation.

An example of a congenic region moved from Dahl R to Dahl S rats is shown in Figure 3. In this case, only the marker Inha was used because the other markers were not available when the work began several years ago. Fortuitously, the congenic strain did move a low BP allele into the S strain decreasing BP 19 mmHg ($P < 0.001$) in the congenic compared with S rats when the rats were fed 2% NaCl diet for 24 days (198). In planning such an experiment with the availability of markers it would have been appropriate to select several markers from D9Rat4 to D9Uia10 in Figure 3.

Congenic strains can be constructed in two ways with a given pair of parental strains. The low-BP strain can be the donor and the high-BP strain the recipient, or vice versa. The main difference between the two is the genetic background. In the Dahl rats, for example, the genetic background of the R rats is not very permissive for expressing BP differences (25, 26, 195) and so most congenic strains using Dahl rats have been made on the S genetic background. On the other hand, successful congenic strains have been made in both directions with SHR and WKY (64).

Once a congenic strain is constructed that includes a QTL, the next logical step is to construct congenic sub-strains with smaller and smaller donor fragments to localize the QTL. One strategy for construction of congenic substrains is shown in Figure 9. The required recombinant chromosomes can be obtained by crossing the congenic strain to the donor strain to produce an $F_1$. Recombinant events in gametes produced by these $F_1$ rats can be recognized by genotyping the markers in the congenic region in rats produced from either an $F_1 \times$ recipient strain cross (i.e., backcross) or in an $F_2$ (i.e., $F_1 \times F_1$ intercross). The $F_2$ cross is preferable because two meioses are scorables in each rat, whereas only one meiosis can be scored in the backcross. In either case, to fix a new recombinant chromosome the rat carrying that chromosome has to be backcrossed to the recipient strain to duplicate the new recombinant chromosome and to obtain male and female rats heterozygous for this chromosome that can be bred to fix the recombinant chromosome in the homozygous state.
Because the original congenic strain produced contains a large segment of chromosome by design (to be sure to include the QTL), it is likely that more than one round of congenic substrains shown in Figure 9 will be required to narrow the QTL location to a workable range. It is also possible (or even likely) that a QTL region contains more than one locus influencing BP. This complicates the construction of congenic substrains as the BP effect seen in the original congenic strain becomes smaller (and thus harder to identify) if it is divided between two (or more) loci in the target region. Fine mapping of the QTL to a resolution of at least 1 cM is required for positional cloning. In mice (and presumably in rats), 1 cM is equivalent to $2 \times 10^6$ bp of DNA. One can expect ~40 genes in such a region, so identification of a base sequence variant that accounts for a (potentially subtle) functional change in a gene is a formidable task.

It is, however, easy to test individual candidate genes in a congenic region for effects on BP by making a congenic strain that includes only the candidate gene and a few centiMorgans of flanking DNA. If such a congenic strain shows no BP effect, then the candidate gene has been eliminated as the gene accounting for the BP QTL. This has been done, for example, with inducible nitric oxide synthase (Nos2) on chr 10. Thus, if a candidate gene is in fact not “the” gene accounting for the QTL, it is easy to rule it out. It is, however, much more difficult to prove that a candidate gene in a small congenic region that is positive for a BP effect is in fact the gene of interest because (1) it becomes more and more difficult to make the congenic strain really small (<0.5 cM) around a candidate gene because a high density of markers in a smaller and smaller region is required and (2) finding recombinants in the small region means screening potentially large numbers of rats (in a 1-cM interval one recombinant is expected for every 100 products of meiosis).

D. Other Strategies for QTL Analysis

In the preceding sections, strategies for defining QTL that are in use for BP analysis were given. In this section other strategies that are either in use in other areas of investigation or that have been suggested on a theoretical basis are briefly given. Additional advanced theoretical considerations are given by Darvasi.

1. Speed congenics

As outlined in section IV C, the construction of congenic strains in rats or mice requires 2.5–3 yr of breeding. Most of this time is spent backcrossing to the recipient strain to dilute out the unwanted donor genome outside of the desired congenic region. The elimination of the donor genome can be expedited by selecting against donor strain alleles at markers strategically placed throughout the genome, at the same time one selects for donor alleles.
at markers in the congenic region. The term speed congenics has been coined for this procedure (136). Theoretical considerations and detailed practical advice on the construction of speed congenics have been presented (160, 270). Such methods allow the production of a congenic strain in 15–18 mo. One caveat is that in moving a large QTL segment of chromosome, recombinant events will occur frequently within the desired region, making it more difficult to find animals positive for the complete donor congenic region and negative for a large proportion of the rest of the donor genome. If, on the other hand, the congenic strain is being made by selection for one specific locus, the procedure should proceed quite well.

The suggestion has also been made (5) that the breeding cycle can be shortened by superovulating (and breeding) 3-wk-old mouse (or rat) pups followed by embryo transfer to mature females for production of the next generation. This might shorten the generation time to 6 wk and the whole congenic procedure to 1 yr. Theoretically, the advantages of superovulation and selection against the donor genome could be combined for the production of a congenic strain in under a year. Although one gains time, the work and technical expertise to implement rapid congenic strain production obviously increases.

2. Interval-specific congenic strains

Darvasi (32) has suggested a theoretically efficient alternate method of producing congenic strains, interval-specific congenic strains (ISCS). Given a pair of parental strains for which the broad intervals containing QTL are known, an $F_2$ population is produced and screened simultaneously for recombinants in all the QTL-containing intervals. In this way, recombinants are efficiently accumulated at all QTL. Congenic strains are then produced from these recombinants by backcrossing to one parental (recipient) strain with selection for the recombinant in one QTL interval and against all the other donor alleles at all other QTL. This is analogous to the procedure for speed congenics, but only the other known QTL are selected against, not the entire genome. After two or three generations of selection, donor segments at the other QTL should be eliminated, and the congenic region is then fixed in the usual manner. Of course, the production of congenic strains at each QTL proceeds in parallel.

3. Congenic strains with phenotypic selection

In theory, it is possible to produce a congenic strain by a series of backcrosses but with selection for the phenotype. For example, one might cross a high and low BP strain and make repeated backcrosses to the low strain, selecting as breeders those rats with the highest BP at each generation. In such a procedure, one would expect to lose the genes with small effects on BP, but the hope is to isolate a strain with a gene(s) having a large effect. Theoretical considerations on how this might work are given by Hill (86).

In fact, such an experiment was tried once with SHR and Wistar/Lewis rats (103), but a successful congenic strain was not obtained. The work is of considerable historical interest because it was one of the earlier attempts at a rational genetic analysis of BP in SHR. Tanase (250) had described Mendelian segregation of BP in crossing SHR $\times$ Donryu rats. Construction of congenic strains on both parental backgrounds appeared successful based on BP segregation in series of backcrosses, but a definitive report on the phenotypically selected congenic strains does not exist, so by inference neither do the congenic strains exist. With the advent of molecular genetic markers, congenic strain construction by phenotypic selection for BP would seem to offer no advantages.

4. Recombinant congenic strains

Recombinant congenic (RC) strains draw on properties of congenic and RI strains (37). Two inbred strains, one of which serves as the donor and the other as the background (recipient) strain, are crossed, and two backcrosses to the background strain are made without selection. At least 20 inbred strains are then developed from this second backcross population by 20 generations of brother-sister mating. Each strain will on average contain 12.5% of the donor genome and 87.5% of the background strain genome. The expectation is that some of the strains will carry different alleles at only one or a limited number of QTL when compared with the background strain. The parental strains chosen to start the RC panel may (or may not) be divergent for a quantitative trait(s) under study. In the case where the parental strains have a similar phenotype for a quantitative trait, they may nevertheless carry different sets of genes resulting in similar phenotypes. Thus, if an RC panel is developed from such strains, genetic information may still be obtained, since QTL will be segregating in the cross. This was the case in at least one RC panel used in a tumor susceptibility study (257).

Each RC strain has to be characterized phenotypically and genotyped at markers throughout the genome to identify regions containing donor alleles (68). Individual RC strains that differ phenotypically from the background strain, or from each other, can be used to produce segregating populations for linkage analysis using microsatellite markers. An individual RC strain is crossed to the background strain to produce an $F_1$ generation and then either backcrossed to the background strain or intercrossed to produce segregating backcross or $F_2$ populations, respectively, for linkage analysis. The advantages of these segregating populations over those produced from the parental strains directly is that only one (or very few) QTL are likely to be segregating in the population, and
this may enhance detection of linkage by reducing the phenotypic variance. Of course, only those markers that are in the donor region of the RC strain used in the formation of a segregating population need be genotyped.

There are at present no RC panels available that would seem relevant to genetic analysis of BP. The use of RC strains of mice in the analysis of tumor susceptibility (61, 167, 257), diabetes mellitus (226), and immunologic phenomena (147) have proven fruitful. The RC strains do of course have the same disadvantages as RI strains, namely, expense of development and maintenance and the fact that one is limited to studying the difference between the two parental strains of each RC panel.

5. Advanced intercross line

In a linkage study of a quantitative trait using an F2 population, a limiting factor on QTL localization is the number of recombination events that occur. An advanced intercross line (AIL) reduces this limitation by accumulating recombination events in a segregating population.

An AIL is produced by first forming an F2 population from two inbred strains. The population is maintained by randomly selecting breeders from the F2 to produce an F3 generation, and repeating this process up to F10. Phenotyping, marker genotyping, and linkage analysis are performed on the F10 population. The accumulation of recombination events in the F10 will effectively expand the genetic map and improve QTL localization. Theoretical considerations (34) suggest that the 95% confidence interval of 20 cM for QTL localization in an F2 is reduced to ~4 cM in the F10 of an AIL. Unfortunately, to produce a useful AIL requires a breeding population of at least 100 individuals at every generation. Darvasi (33) has compared the merits of various approaches to QTL analysis including AIL under a variety of experimental situations.

V. RESULTS OF QUANTITATIVE TRAIT LOCI ANALYSIS OF BLOOD PRESSURE

Molecular genetic analysis certainly proves that BP is polygenic because there is good evidence for many QTL. The objective here is to review the evidence that permits us to place the QTL on genetic maps shown in Figure 10. Most of the QTL placed in Figure 10 either 1) meet the stringent criteria of Lander and Kruglyak (135) for linkage, 2) were confirmed by the construction of a congenic strain showing a significant BP effect, or 3) have been confirmed in at least two independent experiments.

There are several barriers to the accurate placement of QTL in Figure 10. In addition to the inherent statistical inaccuracy of QTL localization, there are often technical differences between experiments that make comparisons among laboratories difficult including 1) different markers used by different investigators, 2) gender effects, 3) dietary NaCl, 4) different strains used which introduce different QTL alleles and/or genetic backgrounds, 5) age at which BP was determined, 6) method of measuring BP, and 7) effects involving systolic, diastolic, mean, or pulse pressure. Where such differences seem important they will be noted, but the philosophy behind Figure 10 is to look for generalities rather than to try to split out subtle differences.

Essentially all BP QTL linkage analysis has been done using adult animals. Because BP increases with age and development, studying adults has the virtue of having the phenotype fully expressed. On the other hand, it has been pointed out that some genetic effects on BP may have developmental components that may not be readily observable without studying BP during growth and development (77, 139, 222). Samani et al. (212) reported that the BP QTL associated with chr 13 was maximally observable in an SHR × WKY cross at 20 wk of age, but not at 25 wk.

A. Historical Perspectives

Linkage analysis of BP using rat models started as early as 1972. Yamori et al. (283) studied the cosegregation of BP in small F2 and backcross populations derived from SHR and Wistar-Mishima rats using as a genetic marker a renal esterase polymorphism that was inherited as a Mendelian trait. Small but significant (in the range of P = 0.01) BP differences were observed among esterase genotypes. I am not aware that this esterase has been mapped, so it is not possible to know if it is in any QTL regions identified in SHR by current linkage analysis. In any case, the concept of the experiments was correct and innovative for the hypertension field.

Rapp and Dahl (191) also published in 1972 a study showing cosegregation of BP with a Mendelian polymorphism in adrenal steroid biosynthesis in Dahl rats. The polymorphism was subsequently shown to be due to differences in steroid 11β-hydroxylase (193). This polymorphism has been studied in depth, and this is reviewed in section vB7. The only other biochemical/physiological trait that was convincingly shown to segregate in Mendelian fashion and to cosegregate with BP involves the response of vascular smooth muscle to micromolar amounts of divalent cobalt (Co2+). Aortic rings from SHR contract in response to 0.6 μM Co2+, but aortic rings from Dahl R rats do not. In an F2(SHR × R) population, modest differences in BP cosegregated with the trait (189). The locus involved has not been further defined or mapped, and thus its relationship to recently defined QTL is unknown.

In 1989, the first use for BP analysis of RI strains derived from SHR and BN was reported by Pravenec et al. (185). Linkage of BP to the major histocompatibility com-
FIG. 10 (part 1)
plex (RT1) on chr 20 was found. In the same paper Pravenec et al. (185) also reported the first use of congenic strains (built around the RT1 complex) to analyze BP. The use of modern techniques for linkage of BP to DNA polymorphism was initiated in 1989 by Rapp et al. (201) using a restriction fragment length polymorphism (RFLP) for the renin gene in Dahl rats. In this case, renin may only be serving as a linkage marker (see sect. vB13). The use of microsatellite markers and genome scans for BP linkage analysis was first initiated in 1991 by Hilbert et al. (85) and Jacob et al. (100) who both located a major BP QTL on chr 10.

Historically, the rat has been the favorite animal for hypertension research. Recently, a genome scan for BP QTL was reported for the mouse. Several QTL are described, and broad regions of rat chromosomes homolo-
gous to the mouse QTL regions are listed (275). At this point, however, a detailed comparison of mouse and rat is not possible because QTL localization in both species is imprecise.

B. Results by Chromosome

1. Chromosome 1

Attention was directed to chr 1 in 1991 when Iwai and Inagami (95) described a gene (the so-called Sa gene) that was expressed more in SHR than WKY kidneys and more in S than R kidneys. The Sa gene (1) is expressed mainly in the renal proximal tubules (178, 284); 2) is located on chr 1 in a region that cosegregates with BP in crosses involving SHR (92, 96, 97, 116, 145, 173, 213), Dahl S rats (66, 70, 78), SBH rats (277), or FH rats (14); and 3) polymorphisms in the Sa gene and its differential expression cosegregate (213). The region of chr 1 that is linked to BP is shown in Figure 10 along with candidate genes in this region. Both Gu et al. (70) and Yagil et al. (277) presented a linkage analysis, suggesting two QTL in this region and both groups localized the two putative QTL to essentially the same positions (see also Fig. 5).

In addition to the Sa gene, other candidate genes in the QTL region(s) include renal kallikrein (and kallikrein-related sequences that are in a cluster on chr 1) that was shown to be linked to BP in the SHR × BN RI strains in 1991 before linkage analysis of the Sa gene (186). Other candidates are the β- and γ-subunits of the epithelial sodium channel (Senn1b and Senn1g, respectively) that are closely linked to each other and to the Sa locus. Sequencing of the genes for sodium channel subunits did not reveal any base changes (69, 88, 123) comparable to those causing a Mendelian form of hypertension (Liddle’s syndrome/pseudoaldosteronism) in humans.

Congenic strains have been developed for relatively large regions of chr 1 all including the region around the Sa locus and all showed significant effects on BP. These congenics included introgression of BN into SHR (206), SHR into BB/OK (a diabetic normotensive rat) (111), WKY into SHR (98), and reciprocal congenics between SHR and WKY (64) and Lewis into Dahl S (66).

Thus there is no doubt that chr 1 contains at least one gene influencing BP, but it is by no means established that the observed differences in Sa gene expression are causating the BP effect. The congenic regions reported so far must contain hundreds of loci, and thus these congenic regions are not very specific to the Sa locus. Lodwick et al. (151) have found using the Milan hypertensive (MHS) and Milan normotensive (MNS) strains that MNS rats have much higher expression of renal Sa mRNA than MHS. The fact that this is opposite to the findings in other hypertensive and control strains has no bearing on the issue because of the polygenic character of BP inheritance; a hypertensive strain can carry minus alleles as some loci, and a normotensive can carry plus alleles at some loci. Lodwick et al. (151) also observed, however, that the Sa locus did not cosegregate with BP in the Milan rat model. Thus it would seem that the Sa alleles causing differential expression were present but that they had no BP effect. Study of congenic substrains suggests that this interpretation is correct and that the QTL actually lies in a chromosomal segment well away from the Sa locus (Y. Saad and J. P. Rapp, unpublished data).

2. Chromosome 2

Evidence first suggesting a BP QTL on chr 2 was found in F2 populations of SHRSP × WKY (100), S × WKY, and S × MNS (40, 44) using the locus for atrial natriuretic peptide receptor/guanylyl cyclase A (Gca). These observations were also confirmed with other F2 crosses GH × BN (79), SHR × BN (221), SHR × WKY (212), and S × R (84) and in the RI strains of SHR × BN (183). Congenic strains introgressing the low BP QTL allele from WKY or MNS into the S background proved the existence of a substantial BP effect (up to 44 mmHg for rats fed 2% NaCl diet for 24 days) in the region around Gca (41). This QTL region of chr 2 is shown in Figure 10 between D2Mgh15 and D2Mit15; in addition to Gca, the region contains loci for Na+-K+-ATPase α1-isofrom (Atp1a1) and calmodulin-dependent protein kinase II-delta (Camk2d).

Lyon hypertensive (LH) rats respond to an intravenous bolus of the dihydropyridine calcium antagonist, py108–068, with a greater acute reduction in BP than do Lyon normotensive (LN) rats. This response was studied in a backcross population, F1(LH × LN) × LH. A QTL (LOD = 4.4) was found on chr 2 that overlaps with the BP QTL between D2Mgh15 and D2Mit15 described above, but no QTL for basal BP was observed (261). Camk2d, which is in this interval, was considered to be a possible candidate gene for the effect of py108–068. The relationship of the py108–068 response QTL to the BP QTL is not established. The data may arise from the coincidental close position of two QTL. It is also possible that if the Lyon backcross population were challenged with increased salt intake that an effect on BP per se would also be observed. The pharmacological effect of py108–068 was specific in that the QTL observed had no effect on the acute responses to a ganglionic blocking agent (trimetaphan) or an angiotensin II subtype 1 receptor antagonist, losartan.

The role of the Na+-K+-ATPase α1 (Atp1a1) as a candidate gene in the QTL region between D2Mgh15 and D2Mit15 is controversial. As noted above using Dahl S rats F2 populations derived from S × WKY and S × MNS yielded BP QTL in the Atp1a1 region that was confirmed by the construction of congenic strains substituting WKY or MNS alleles into the S background (40, 41, 44). An F2(S
The most permissive population was an Atp1a1 allele inserted (random placement) into the S rat. There was a dramatic reduction in BP in the transgenic rats compared with S rats. No evidence was presented, however, to prove that the reduction in BP was due to special properties of the transgene as opposed to just overexpression of Na⁺-K⁺-ATPase α₁ as a result of having both the endogenous and transgenes present in the same rat.

There is reasonable evidence for a second BP QTL on chr 3 between D2Mit5 and D2Mgh24 (Fig. 10). A QTL reaching the suggestive level of significance was seen in F₂ populations derived from LH × LN (49), SHRSP × WKY (27), S × LEW (66), and S × MNS (40). In the Lyon rat cross, both systolic and pulse pressure yielded evidence for a QTL. The gene for angiotensin II receptor 1b (Agtr1b) is located. Kato et al. (106) also presented evidence for linkage of BP to the distal end of chr 3 in an F₂ (SHR × WKY) cross (224). This cardiac mass QTL was independent of BP in the population studied. It does fall in the general region of the proximal chr 3 BP QTL described above, but because the regions involved are large and poorly defined, it is not possible to know without further data if they reflect the effects of the same genetic locus (or loci).

4. Chromosome 4

Two F₂ populations derived from SHR × WKY (108) or SHR × BN (221) and one backcross F₁ (SHR × BB/OK) × BB/OK (117) all provided consistent evidence for a BP QTL in the region of neuropeptide Y (Npy) (Fig. 10). This region also includes the β-adducin subunit gene (β-add) (253) that has been implicated in BP changes in MHS rats (9). See chr 14, which contains the β-adducin subunit, for a more complete description of the relationship of adducin to BP. Evidence for an additional BP QTL on chr 4 near interleukin-6 (IL-6) has been given using the RI strains derived from SHR × BN (183).

5. Chromosome 5

Attention was first brought to the QTL for BP on chr 5 by Deng et al. (39) using endothelin 2 (Edn2) as a candidate gene. Edn2 cosegregated with BP in an F₂ (S × LEW) population, and this QTL was subsequently more accurately mapped to the region of chr 5 shown in Figure 10 by linkage and construction of a congenic strain (66). Another candidate gene in this region is cytochrome P-450A2 which catalyzes production of 20-hydroxyeicosatetraenoic acid (20-HETE) from arachidonic acid. Reduced production of 20-HETE in Dahl S rats is thought to be involved in the elevated Cl⁻ transport in the loop of Henle in S rats and the marker for P-450A2 (D5Rjrl) cosegregated with BP in salt-loaded F₂ (S × LEW) (240) and F₂ (SHR × BN) (241). Atrial natriuretic peptide (Anf) and brain natriuretic peptide (Bnp) were also found to be linked to BP in studies of F₂ (SHR × WKY) (290, 291), implying existence of a second QTL on the distal end of chr 5 near Anf (Fig. 10).
6. Chromosome 6

No QTL for BP have been reported on chr 6.

7. Chromosome 7

Adrenal steroid 11β-hydroxylase (Cyp11b1) catalyzes both the 18- and 11β-hydroxylation of 11-deoxycorticosterone (DOc) to form 18-hydroxy-11-deoxycorticosterone (18-OH-DOC) or corticosterone, respectively. Rapp and co-workers (26, 191) found that S rat adrenals produce more 18-OH-DOC than R rats and that the altered steriodogenic pattern segregates in Mendelian fashion and cosegregates with BP. Enzymatic studies (193) showed that genetic variants in 11β-hydroxylase caused the altered steriodogenesis. Subsequently, it was found that position 127 was different at a single base in five locations in the coding region causing amino acid substitutions at amino acids 127, 351, 381, 384, and 443 (26, 162). By constructing chimeric cDNA for 11β-hydroxylase of S and R rats and expressing these in COS-7 cells, Matsukawa et al. (162) showed that substitutions at 127 and 351 did not alter the strain-specific steriodogenic pattern but that positions 381, 384, and 443 were associated with the strain-specific patterns. Structural arguments largely ruled position 443 out as important, and it was concluded that amino acid substitutions at 381 and/or 384 were the cause of the strain-specific steriodogenic pattern. This makes sense because these residues are thought to be in or near the substrate recognition site. More recently, this interpretation has been confirmed using site-directed mutagenesis and expression in an Escherichia coli system. Nonaka et al. (174) showed that the variants at amino acids 381 and 384 are both required, and are almost sufficient, for full expression of the difference in 18-OH-DOC production characteristic of S and R rats.

A congenic strain was constructed by introgressing the R allele into the S strain using only genetic markers for Cyp11b1. This resulted in a congenic strain that of course included Cyp11b1 plus ~22 cM of flanking DNA; this region is shown on Figure 10. The congenic strain had a BP dramatically lower than S rats (20 mmHg on 2% NaCl diet for 24 days and 63 mmHg on 4% NaCl diet for 24 days in males). Average survival on 4% NaCl diet was markedly increased in the Cyp11b1 congenic strain compared with S (112 ± 25 vs. 40 ± 3 days in males), and the difference in survival was accounted for by the differences in BP (23).

Cyp11b1 is a candidate gene for which a strong argument can be made that it is the gene responsible for the BP QTL (192). The steroid 18-OH-DOC is mildly hypertensinogenic, and it is produced in the zona fasciculata of the adrenal under the control of ACTH. Increased dietary salt suppresses aldosterone production from the adrenal zona glomerulosa, which is under feedback regulation by the renin-angiotensin system. On a low-salt diet, aldosterone dominates the total mineralocorticoid status of the rat. On high-salt diet, however, aldosterone is suppressed but 18-OH-DOC is not, and 18-OH-DOC contributes significantly to the net mineralocorticoid status of the rat. Because S rats have increased plasma 18-OH-DOC concentration compared with R rats (162, 192, 200), they can be expected to have a mineralocorticoid-induced component of their hypertension on a high-salt diet. This can, of course, account (192) for the genotype × dietary salt interaction (31, 194) seen between S and R rats. In spite of such arguments, the role of Cyp11b1 needs to be further evaluated by construction of a congenic strain containing Cyp11b1 and much less flanking donor chromosome than exists in the congenic strain noted above.

Weak evidence for a BP QTL in an F2(S × LEW) population was seen ~20 cM from Cyp11b1 (66). If real, this QTL is probably different from the QTL associated with Cyp11b1 because of the distance between them.

8. Chromosome 8

Several independent studies indicate the existence of a BP QTL on chr 8, all in the region shown in Figure 10. Linkage analysis in F2(SHR × BN) (221), F2(SHR × WKY), F2(Slw × WKY) (249), and F2(S × LEW) (66) all provided suggestive evidence for a QTL. Fortuitously, this region contains the gene for polydactyly-luxate syndrome (Lx) in the rat. In studying limb morphogenesis, a congenic strain had been made placing the Lx region from BN on the SHR background (119). It was subsequently shown that this congenic strain (SHR.Bn/Lx) had a lower BP than SHR by ~20 mmHg (120). The region contains some speculative candidate genes, the brain dopamine receptor (Drd2), the renal epithelial potassium channel (Kcnj1) (120), and a smooth muscle specific protein Sm22 (113).

9. Chromosome 9

Takami et al. (249) first reported a weak linkage of BP to chr 9 around D9Mit2 in an F2(SHR × WKY) population. Using a marker for the α-subunit of inhibin (Inha), Rapp et al. (198) recently reported strong linkage (LOD = 5.0) to BP in an F2(S × R) population (illustrative data given in Figs. 2 and 3). A congenic strain was constructed introgressing a 21-cM segment of R chr 9 into the S strain; the congenic strain had a significantly lower BP (19 mmHg, P < 0.0001, 2% NaCl diet 24 days) than S, proving the existence of a BP QTL. The congenic region on chr 9 is shown in Figure 10 (see also Fig. 3). A possible candidate gene, anion exchange protein 3 (Ae3 also called Sla4a3), is included in this region (Fig. 10).

10. Chromosome 10

In 1991, two groups (85, 100) using the identical F2(SHRSP × WKY) population, which had been pheno-
typed for BP at the University of Heidelberg, found strong evidence for BP linkage to a region of chr 10 near angiotensin converting enzyme (Ace). Corroborating evidence for this linkage has been reported in \( F_2 \) populations derived from \( S \times MNS \) (44, 45), \( S \times LEW \) (66), \( S \times BN \) and \( S \times WKY \) (106), SHRSP \( \times WKY \) (171, 172), SHR \( \times WKY \) (289), SHR \( \times BB/OK \) (116), and GH \( \times BN \) (79).

Congenic strains substituting either segments of chr 10 from LEW (66) or MNS (51) into the S background proved the existence of a major BP QTL in the region around Ace. A series of congenic strains using MNS donor chr 10 segments was particularly useful. The region defined that contains the QTL (51) is shown in Figure 10, although this was still a large segment of 30–35 cM. A congenic strain was also produced utilizing a small (~2.5 cM) segment containing the candidate gene inducible nitric oxide synthase (Nos2). This congenic strain had no BP effect, eliminating Nos2 as the causative BP gene in the comparison of S and MNS (51).

Kreutz et al. (122) has reported on the existence of three substrains of WKY at the University of Heidelberg, designated WKY\(_{HD-0}\) and WKY\(_{HD-1}\), that differ in BP by 6 mmHg. This was thought to be due to a difference in a 6-cM fragment roughly between D10Mit2 and D10Mgh8. This segment is also indicated in Figure 10, but it is out of the region proven to contain a major QTL on chr 10 by construction of a congenic strain and supported by multiple linkage studies. The Heidelberg WKY substrains were thought to have arisen by genetic contamination from SHRSP.

There was a major difference in the linkage data of crosses between these two WKY substrains with SHRSP. In \( F_2 \) (SHRSP \( \times WKY_{HD-0} \)) there is a strong BP linkage to Ace, but in \( F_2 \) (SHRSP \( \times WKY_{HD-1} \)), no such linkage is observed (121, 122). It was interesting that in \( F_2 \) (SHRSP \( \times WKY_{HD-1} \)) large differences in plasma Ace activity were almost completely determined by the Ace locus (121). The simplest explanation for these data is that SHRSP and WKY\(_{HD-1}\) have functionally identical alleles at the QTL but have functionally contrasting alleles at the Ace locus. From this interpretation it follows that Ace is not likely to be the gene responsible for the major QTL on chr 10.

The region of rat chr 10 containing the major QTL is homologous to a region of human chr 17q in which affected-sib-pair analysis provides strong statistical evidence for a BP QTL (104). The region is also a candidate region for human familial hyperkalemia and hypertension (pseudohypoaldosteronism type II, Gordon’s syndrome) (157).

11. Chromosome 12

No BP QTL have been reported for chr 11.

12. Chromosome 12

One report (106) shows linkage of BP to chr 12 in an \( F_2 \) (S \( \times WKY \)) population.

13. Chromosome 13

Linkage of BP to chr 13 was reported by Rapp et al. in 1989 (201) using the renin locus (Ren) as a candidate gene in an \( F_2 \) (S \( \times R \)) population. The result was duplicated (196), and it was shown that genetic background was important in detecting the chr 13 QTL in that linkage was more easily demonstrated in a backcross to S than in \( F_2 \) or backcross to R populations (202). Linkage of BP to chr 13 was amply confirmed in other crosses: \( F_2 \) (SHR \( \times WKY \)) (212, 243, 287), \( F_2 \) (SHR \( \times LEW \)) (132), RI strains derived from SHR and BN (187), and \( F_2 \) (LH \( \times LN \)) (49, 105).

Ren is an obvious candidate locus, and it was the marker that brought attention to chr 13, but is Ren likely to be the locus accounting for the chr 13 QTL? Several arguments suggest that it is not. In the comparison of Dahl S and R rats, the S rat renin allele was associated with increased BP in linkage analysis. S rats, however, have low plasma renin (200), which makes little sense if renin is part of a mechanism causing higher BP. Of course, low renin as a physiological response to higher BP makes sense since renin is well known to be suppressed by increased BP. Similar arguments were made by studying kidney renin concentration in an \( F_2 \) (LH \( \times LN \)) cross where no evidence was found for a renin genotype-dependent phenotypic difference in the renin-angiotensin system that might account for linkage of the renin locus to BP (105). Moreover, it was possible to distinguish 7 renin alleles among 27 inbred rat strains, and it was found that LH and R rats apparently carry the same renin allele (r) and that LN and S carry the same renin allele (s) (196). In \( F_2 \) populations, the r allele from LH rats cosegregated with higher BP, and the s allele from LN rats cosegregated with lower BP (49). This is exactly the opposite of the case with Dahl rats where in an \( F_2 \) population the r allele was associated with lower BP and the s allele with higher BP (196). This implies that the high BP QTL allele is on the same chromosome with the s renin allele in S rats but that in the LN rats the r allele is on the same chromosome with the high BP QTL allele. Thus Ren is probably not the QTL on chr 13 detected in linkage analysis of S and R rats (196). Finally, DNA sequences of the coding and 5’-flanking region of the renin alleles of S and R rats were identical (2).

A congenic strain introgressing the R rat renin allele and flanking DNA into the S background was constructed and shown to have a lower BP than S rats (24 mmHg, 24 days on 2% NaCl diet) as would be expected based on the linkage results. A congenic substrain made from the original congenic and which included Ren did not differ in BP.
from S rats. Thus it was concluded that Ren is not the locus accounting for the BP effect of chr 13, and the QTL was localized to the segment between Syt2 and D13Uia3 (see Fig. 10) (292).

Two other congenic strains using S and R rats have been developed. St. Lezin et al. (208) transferred an S segment of chr 13 onto the R background, and Jiang et al. (102) transferred an R segment onto the S background. In both cases, the region transferred between strains does not include the region defined above by Zhang et al. (292) and does include the Ren locus. In both cases, the BP change associated the higher BP with the R chromosomal segment. This is the opposite of what would be expected based on the linkage analysis in S and R rats. The minimal region defined by Jiang et al. (102) is between D13N1 and Syt2 (see Fig. 10). One possible interpretation of all the congenic data is that there are two QTL on chr 13 that are in repulsion, i.e., the S rat chr 13 has a plus BP allele at a QTL between Syt2 and D13Uia3 and a minus BP allele at a QTL between Syt2 and D13N1 (see Fig. 10) with the former QTL dominating the net effect likely to be seen in linkage analysis. A problem with the studies of Jiang et al. (102) and St. Lezin et al. (208) is that both studies utilized Dahl S rats that were genetically contaminated by the commercial supplier Harlan Sprague-Dawley. Despite the fact that both investigators addressed this issue by typing multiple genetic markers to try to establish the genetic integrity of their experiments, serious questions remain as to the validity of their conclusions with regard to the S rat chr 13. After all, using authentic S rats, the congenic substrains on chr 13 constructed by Zhang et al. (292) covering the regions studied by Jiang et al. (102) and St. Lezin et al. (208) yielded no significant BP effect. This convoluted situation emphasizes the importance of insisting on genetic identity of standard animal models if any sense is to be made of experiments done in different laboratories. In Figure 10, the QTL region for BP shown includes all of the reported congenic strains with significant BP effects and unfortunately does not provide a satisfactory localization.

A congenic strain introgressing the renin locus of BN into SHR has also been constructed (207). Because only minor BP differences were detected that were in the direction opposite of that expected based on the linkage analysis, the authors concluded that the chr 13 QTL must be outside the region moved (Ren to D13N1).

14. Chromosome 14

Attempts to explain cellular differences in Na\(^+\) transport between MHS and MNS strains (57) led to the description of strain differences in adducin (58, 59, 211). Adducin is a cytoskeletal protein composed of an \(\alpha\beta\) heterodimer. \(\alpha\)-Adducin (\(\alpha\)-add) is on chr 14, and \(\beta\)-adducin (\(\beta\)-add) is on chr 4 (253). An A to T transversion in \(\alpha\)-add results in tyrosine at amino acid 316 in MHS and phenylalanine in MNS, and a G to A transition in \(\beta\)-add results in arginine at amino 529 in MHS and glutamine in MNS (9). Both of these amino acid substitutions alter the degree to which the adducin subunits are phosphorylated (9), and thus there is a reasonable chance that these changes alter function. Recent evidence using cell-free systems and transfected rat renal epithelial cells shows that the adducin isoforms do modulate actin assembly and Na\(^+-\)K\(^+\) pump activity (254).

A linkage analysis in an \(F_2\)(MHS × MNS) population showed a modest effect of \(\alpha\)-add on BP, but no effect of \(\beta\)-add. There was, however, a substantial interaction (\(P = 0.005\)) of \(\alpha\)-add and \(\beta\)-add loci on BP. When \(\beta\)-add was homozygous for the MNS allele, there was no gene-dosage effect of \(\alpha\)-add on BP, but when \(\beta\)-add was either heterozygous or homozygous for the MHS allele, then there was a significant gene-dosage effect of \(\alpha\)-add on BP (9).

The work on adducin represents a very nice progression of logical experiments from an intermediate phenotype (Na\(^+\) transport) to finding genetic polymorphisms in candidate genes. Still lacking, however, are congenic strains involving the adducin subunit loci with minimal flanking DNA to corroborate the other data.

15. Chromosome 15

No QTL for BP have been reported for chr 15.

16. Chromosome 16

Suggestive evidence was reported for linkage of BP to chr 16 in the vicinity of D16Mit2 in \(F_2\)(SHR × BN) (221) and \(F_2\)(S × LEW) (66) (Fig. 10).

17. Chromosome 17

Suggestive evidence for linkage of BP to the middle of chr 17 was reported in \(F_2\)(S × LEW), but a congenic strain made in this region introgressing the LEW region into S rats showed no BP effect (66). Nevertheless, there may well be a BP QTL on chr 17 because in another study using \(F_2\)(SBH × SBN) a significant linkage to BP in exactly this same region was found (277) (Fig. 10). It is possible that the congenic strain reported by Garrett et al. (66) missed the QTL because it did not span the entire target region due to a lack of genetic markers available during construction of the congenic strain. The angiotensin receptor 1a (Atr1a) is in the putative QTL region.

18. Chromosome 18

A suggestive linkage of BP near one end of chr 18 around D18Mit7 was reported originally by Jacob et al. (100) in \(F_2\)(SHRSP × WKY). This was not confirmed in another \(F_2\)(SHRSP × WKY) population (122), but the
subline of WKY used in the two populations was different; this emphasizes again the need for a high degree of genetic integrity of breeding stocks. Garrett et al. (66) also found suggestive linkage to BP in $F_2(S \times LEW)$ near the same end of chr 18, but the regions are too poorly defined to know if they represent confirmation of the same QTL.

Kovács et al. (116) reported a very strong BP QTL on chr 18 (LOD = 19) in an $F_2$ cross of SHR and BB/OK (spontaneously diabetic) rats. The QTL was poorly localized however, and the region indicated in Figure 10 is a rough approximation. This QTL would appear to be at the opposite end of chr 18 from the one putative QTL described above.

19. Chromosome 19

In an early study to test the angiotensinogen locus ($Agt$) on chr 19 for linkage to BP, no linkage was found in an $F_2$(SHRSP $\times$ WKY) population (89). In a subsequent study, Agt gave a modest linkage signal to BP in an $F_2$(SHR $\times$ WKY) population (149). In a study using RI strains derived from SHR and BN, Agt did not cosegregate with BP, but D19Mit7, $\sim$10 cM from Agt, was linked to BP with modest significance (183). A congenic strain introgressing a region of chr 19 from BN encompassing Agt did significantly lower BP, indicating a QTL in the region shown in Figure 10 (210).

20. Chromosome 20

Interest in chr 20 has been restricted to the major histocompatibility complex $RT1$. This probably stems from the early study in 1989 by Pravenec et al. (185) when genetic markers were not available in large numbers, but the $RT1$ complex could be genotyped immunologically. In this study the RI strains between SHR and BN were used to show linkage of this study the RI strains between SHR and BN were used from the early study in 1989 by Pravenec et al. (185) when histocompatibility complex $RT1$ was confirmed by a congenic strain (SHR-RT1.N) in which the BN was not challenged by a congenic strain (BB.1K) moving the chr X (89). A congenic strain moving the chr X region of the SHR indicated by Hilbert et al. (85) onto the BB/OK background was successful in raising the BP on the congenic strain compared with BB/OK (111); this region is indicated in Figure 10. In Sabra rats, $F_2$(SBH $\times$ SBN) showed BP linkage to a broad segment of chr X (including the region targeted above, Ref. 278). One important candidate gene, the angiotensin type 2 receptor ($Agtr2$), has been shown to fall well outside the QTL region of chr X (114).

22. Chromosome Y

Ely and Turner (56) first reported evidence for a Y-chromosome effect on BP. $F_1$ males of a WKY female $\times$ SHR male cross had higher BP than $F_1$ males from the reciprocal cross of SHR female $\times$ WKY male. To differentiate the potential effects of the X and Y chromosomes in $F_1$ rats, $F_2$ males derived from these reciprocal-cross $F_1$ rats were studied. In these $F_2$ populations (SHR grandfather vs. WKY grandfather), X chromosomes from either SHR or WKY will segregate, and their effects will cancel out, but the Y chromosome in each population will be unique (either SHR or WKY). The BP of $F_2$ males was compatible with an Y-linked effect on BP. Reciprocal Y-consomic strains (SHR Y chromosome on WKY background and WKY Y-chromosome on SHR background) were constructed and confirmed a BP effect of the Y chromosome (54).

One subsequent study (260) could not confirm the Y-chromosome effect between SHR and WKY. In another study, however, an elevating effect on BP of the SHR Y chromosome was seen in crosses derived from SHRSP and WKY (36). Of course, the fact that various SHR and WKY stocks are not identical could easily account for such inconsistencies.

The Y chromosome contains very few known genes, so the mechanism whereby it may influence BP will be hard to define. It has been suggested (55) that the SHR Y chromosome may accelerate increases in testosterone secretion during puberty.
VI. SEX EFFECTS ON BLOOD PRESSURE
QUANTITATIVE TRAIT LOCI

Most of the linkage analysis for BP has been done on male rats, probably because males develop hypertension faster than females, and they are larger, which expedites BP measurement. Thus much of the initial screening done for BP QTL has missed any effects of sex per se on expression of a QTL. Dealing with the two sexes complicates the experimental design for QTL analysis. One is really interested in interactions between a QTL and sex. The readily available computer programs for QTL linkage analysis do not accommodate a factorial design, much less an analysis of interactions. Most authors have just analyzed the sexes separately for QTL and commented on any differences, or they have used factorial analysis of variance (sex × genotype) at specific genetic markers of interest.

In studies with Sabra rats, Yagil et al. (277) reported that of the two QTL described on chr 1, one QTL was seen only in males and the other only in females. In this study, it was also claimed that the QTL on chr 17 was observable only in females. The same group (278) has also described BP QTL on chr X that were seen only in females. Using populations derived from GH and BN rats, Harris et al. (79) claimed sex specificity for the QTL on chr 2 near the marker Gca (linkage observed only in males) and the QTL on chr 10 near Ace (linkage observed only in females). Clark et al. (27) presented suggestive evidence that the BP QLT on the proximal end of chr 3 in an F2(SHRSP × WKY) population was observable only in males.

VII. INTERACTIONS AMONG BLOOD PRESSURE
QUANTITATIVE TRAIT LOCI

The models for statistical analysis of QTL usually assume additive effects as a first approximation. It is clear, however, that interaction among alleles at different QTL (epistasis) is likely (63), and numerous examples of epistasis have been reported in Drosophila, plants, and mammals for quantitative traits. With regard to BP, it was obvious that genetic background had important effects. In crosses involving Dahl S and R rats, the higher the proportion of S genes in the background, the more significant the linkage to BP for the QTL on chr 3 (25), chr 7 (26), or chr 13 (202). The genetic background was altered in these studies by studying populations F1(S × R) × R, F2(S × R), and F1(S × R) × S that have genetic backgrounds composed of S alleles in proportions of 25, 50, and 75%, respectively. Such results imply genetic interactions but obviously do not define which loci are interacting.

A major interaction on BP was reported by Deng and Rapp (44) between the QTL on chr 2 and chr 10 in an F2(S × MNS) population. A double congenic strain introgressing minus alleles on chr 2 and chr 10 into the Dahl S rat background was subsequently constructed. Comparison of the BP response to high-salt diet of the double congenic, the two single congenics with minus alleles on chr 2 or chr 10 (on the S background), and S rats, revealed a strong interaction accounting for 24 mmHg and 79 mg heart weight (199) (Fig. 11). An interaction involving these same areas of chr 2 and chr 10 was seen for heart weight in an F2 cross of GH rats and BN (79).

An especially interesting interaction between the loci for adducin α- and β-subunits was described under chr 14 above. Additional interactions were subsequently observed in an F2(S × LEW) population (66). One prominent interaction involved the major QTL on chr 10 interacting with a region of chr 4 around D4Mit17, which is possibly identical to the QTL on chr 4 reported in other crosses. Another interaction in F2(S × LEW) involved the region of chr 2 around the angiotensin receptor 1b (Agtr1b) and the central part of chr 3. The interacting regions of chr 3 or chr 4 in the F2(S × LEW) cross were not detected as

![Graph showing an interaction of chr 2 and 10 on BP.](http://physrev.physiology.org/)
QTL by themselves but were only prominent as interacting regions. It is, therefore, interesting that these regions of chr 3 and chr 4 do appear as QTL in other crosses (discussed above under individual chromosome and indicated as QTL in Fig. 10).

VIII. QUANTITATIVE TRAIT LOCI FOR TRAITS RELATED TO BLOOD PRESSURE

Important consequences of hypertension are cardiac hypertrophy, stroke, and renal damage. It is reasonable to expect the BP QTL will colocalize with the sequelae to hypertension. It is also reasonable to hypothesize that genetic loci could influence each of these traits independently of BP and/or alter susceptibility of end organs to damage by hypertension.

A. Cardiac Hypertrophy

Blood pressure QTL are essentially always associated with effects on heart weight as the heart hypertrophies in response to chronically increased BP. A priori it is also reasonable to expect that loci exist that influence heart weight independently of BP or body weight. The claim has been made (92) that chr 2 near D2Mgh15 (Fig. 10) influences heart weight independently of BP in $F_2$ (SHR × Donryu) rats. This is at the edge of a well-established BP QTL that makes this claim more difficult to interpret without some additional evidence (e.g., a congenic strain for heart weight independent of BP and body weight). Suggestive evidence is available for linkage of heart weight to chr 14 near D14Mgh3 (27) and for chr 17 near the dopamine 1A receptor (Drd1A) (183) (Fig. 10). In these regions there are no known closely linked BP QTL. In $F_2$ (SHR × WKY) rats, the 27-kDa heat shock protein locus ($HSP_{27}$) cosegregated with left ventricular weight but not with BP (73); $HSP_{27}$ was stated to be on chr 12 by Hamet et al. (73) without data or reference. With the use of the Lyon LH and LN rats as studied in a reciprocal backcross design, there was evidence for an effect of the X chromosome on left ventricular weight independent of BP (259).

B. Stroke

The SHRSP is clearly an interesting model in which to analyze stroke. In an initial analysis (91) using $F_2$ (SHRSP × WKY), suggestive evidence for linkage to stroke was obtained on chr 4 in the region of D4Mgh7 and D4Mgh8. This region is distinct from the BP QTL on chr 4 (Fig. 10), and these markers were not linked to BP in this study. The stroke phenotype used was brain weight, presumably reflecting brain edema in rats that had clinical signs of stroke. The study produced only 9 rats with stroke out of 107 $F_2$ rats studied and thus has very limited statistical power.

In another study, Rubattu et al. (204) used an $F_2$ population derived from SHRSP and SHR. The idea was to minimize the BP difference between the parental strains but yet create a hypertensive genetic background that would be permissive for stroke. The phenotype studied was days to clinical evidence for a stroke (stroke latency); strokes were confirmed by brain histology. Apparently all 220 $F_2$ rats did have a stroke by 400 days of age. Strong linkage of stroke latency to three chromosomal regions was obtained. On chr 1 a region centered around D1Mit3 linked to stroke but not to BP in this population. This region is, however, exactly at the center of the region of chr 1 associated with one or more BP QTL. Linkage for stroke latency was also seen on chr 4 around D4Mgh16. Linkage to BP was not observed around D4Mgh16 in the $F_2$ population studied, although this stroke QTL region overlaps with a previously described BP QTL region on chr 4 (Fig. 10). A third stroke latency QTL was identified in the $F_2$ (SHRSP × S) population by Rubattu et al. (204) on chr 5 around the locus for atrial natriuretic factor (Anf), but again, no BP effect in the test population was observed around Anf. Zhang and co-workers (290, 291), however, did report linkage of BP to Anf in $F_2$ (SHR × WKY). In the study of Rubattu et al. (204), epistatic interaction between the stroke latency QTL on chr 1 and 5 was also observed.

Jeffs et al. (101) have reported a stroke QTL on chr 5 using an $F_2$ (SHRSP × WKY) population. Here the phenotype was brain infarct size following occlusion of the middle cerebral artery (MCA), such infarct size being larger in SHRSP than WKY. A QTL was identified on chr 5 with an exceptionally strong linkage (LOD = 16.6, accounting for 67% of the total variance) in a region that colocalizes exceptionally well with the BP QTL observed in studies with Dahl S rats (66). It should be noted in the study of Jeffs et al. (101) that the Anf locus is misplaced in the center of their QTL; its actual location is near the telomere as shown in Figure 10 from consensus linkage maps (13, 42, 204) and by fluorescent in situ hybridization (12).

How many BP/stroke QTL are on chr 5? Unpublished data on congenic strains (J. Rapp and M. Garrett, unpublished data) localize the BP QTL on chr 5 to a much smaller region in the middle of the larger BP QTL region indicated in Figure 10, which is based on the published congenic strain (66). Thus it would appear that this BP QTL colocalizes with the QTL for stroke induced by MCA occlusion described by Jeffs et al. (101) but that the stroke latency QTL of Rubattu et al. (204) at the region around Anf is a separate entity. This interpretation of two separate stroke QTL on chr 5 is supported by the fact that $I$ the stroke phenotypes utilized (stroke latency vs. in-
farct size after MCA occlusion) could have different underlying mechanisms; and 2) in the study of Rubattu et al. (204) the SHRSP allele was associated with stroke protection (increased latency), whereas in the work of Jeffs et al. (101) the SHRSP allele was associated with stroke enhancement (larger infarct size).

The fact that the BP QTL identified on chr 5 in an F2(S × LEW) population colocalizes with the stroke infarct size in F2(SHRSP × WKY) of course does not prove that they are the same locus because the intervals defined are still too large. An additional paradox is that in the F2(SHRSP × WKY) population linkage of BP to the stroke region was not observed. In many studies involving SHRSP, linkage to BP was most easily observed on high salt intake; in the study of Jeffs et al. (101), the F2(SHRSP × WKY) were fed normal rat food, which might reduce the chance to observe a BP effect. In the study of Garrett et al. (66), the F2(S × LEW) rats defining the BP QTL on chr 5 were raised on a high-salt diet. A unifying speculation could be that a vasoconstrictor response influenced by a locus on chr 5 at the renal or systemic level responds to salt intake in the Dahl rat model and also responds to local brain ischemia increasing infarct size.

C. Renal Damage

It is well known that renal damage in rats occurs in response to hypertension induced experimentally by a variety of nongenetic manipulations (e.g., deoxycorticosterone plus salt, renal artery constriction). Thus it can be anticipated that BP QTL should also be associated with renal damage. However, it is also reasonable to anticipate that genes exist that increase or decrease renal damage in response to a given hypertensive state. Churchill et al. (20) used a renal transplantation model between histocompatible BN and SHR in which genetically different kidneys in the same host were exposed to the same hypertensive environment. It was concluded that the BN kidney is inherently more susceptible to hypertension-induced renal damage than the kidney of SHR.

A linkage study in an F2(FHH × ACI) × FHH backcross population (14) yielded two QTL associated with renal sclerosis and/or proteinuria. Both QTL mapped to rat chr 1. One locus, called RF-2 for renal failure-2, is in the same region of chr 1 as BP QTL seen in several other populations. The colocalization of QTL for BP, stroke, and renal damage on chr 1 is impressive (Fig. 10) and is presumably due to the damaging effects of hypertension on the vasculature, resulting from the BP QTL. A second locus for renal damage, RF-1, was also found on chr 1, but in this case, the locus was clearly different from the region containing one or more BP QTL (14) (Fig. 10).

IX. PERSPECTIVE

Localization of QTL by linkage analysis has undergone remarkable advances with the advent of PCR-based, highly polymorphic, genetic markers and the development of sophisticated methods of statistical analysis. There is credible evidence for at least one BP QTL on every rat chromosome except chr 6, 11, and 15. Localization of QTL by genetic linkage is, however, nowhere near precise enough to allow identification of genes controlling BP by positional cloning. Moreover, it is anticipated that the large ill-defined chromosomal segments identified for controlling BP may often contain more than one locus influencing BP, or may be false positives. An additional challenge is defining interactions among QTL, especially when one or more loci involved in epistasis are not also detectable as independent QTL.

The goal of QTL analysis of BP in the rat is identification of the loci controlling naturally occurring genetic variation in BP. Alleles at these loci need to be characterized with regard to changes in their DNA sequence and the function of their protein products. Such changes of function are likely to be quantitative and more subtle than the drastic changes (e.g., complete loss of function) often associated with recessive Mendelian diseases. In two cases where a QTL has potentially been identified, however, one represents subtle functional variants and the other a drastic loss of function. 11β-Hydroxylase variants in Dahl rats (see chr 7 above) represent subtle changes in DNA and amino acid sequence that result in modest quantitative alteration of function. The gene Cd36 (fatty acid translocase) is probably responsible for a component of the insulin resistance phenotype in SHR (1). In this case, the SHR allele was created by a deletion event resulting in a nonfunctional chimeric transcription unit.

How can identification of the loci causing BP variation proceed? A first step is fine mapping of a QTL to a resolution of <1 cM by the use of congenic strains. It is feasible to create a minimal congenic strain containing an introgressed chromosomal segment of <1 cM. One centi-Morgan in terms of linkage represents an interval between genetic markers where 1 recombinant event is observed in every 100 products of meiosis. Because two meiotic events are observable in F2 rats derived from crossing a congenic strain with its background (recipient) strain, screening 500 rats allows evaluation of 1,000 meiosis. On average, 10 recombinants are expected in a target region of 1 cM in 1,000 meiosis. Screening DNA from tail biopsies of 500 rats is quite feasible using PCR-based microsatellites. The ultimate resolution of fine mapping of a QTL using congenic substrains is, however, more likely to depend on the marker density needed to observe the exact position of crossover events in the target chromosomal region, and on the nonrandom nature of recombi-
nation, than it is on the number of rats that can be screened.

Although marker density has recently improved drastically for the rat, the availability of a very dense map in a target region <1 cM is still problematic. It should, however, be possible to prepare a YAC contig across such a target region for the dual purpose of obtaining additional microsatellite markers and to provide cloned material for gene identification and sequencing projects in the target region. Given a high density of markers in a target region, the QTL fine mapping may, however, still be limited by the fact that (in mice and presumably in rats) chromosomal recombination events are nonrandom below 0.2 cM (231). This means that recombination sites tend to be clustered; there will be 0.2-cM regions in which crossing over just does not occur. This limits how finely the region can be divided by recombination and thus how small the congenic region can be made. Nevertheless, QTL localization by fine mapping with congenic strains is feasible down to the 0.3–0.5 cM range.

Once a QTL allele is localized to a very small congenic region, there are three approaches that can be taken: 1) search for candidate genes, 2) look for differential expression of genes, and 3) sequence the region. Known genes in the region can be sought from rat genetic maps and/or from conserved syntenic regions of human or mouse (28). Two useful web sites for comparative mapping are www.otsuka.genome.ad.jp/ratmap and www.ncbi.nlm.nih.gov/Omin/. If logical candidate genes are present, then they can be studied for allelic variants and functional differences between the congenic and background strain.

It will be of interest to look for differences in expression of the genes in the minimal congenic region between the minimal congenic and the background strain. It can be speculated that at least some causative genes will be differentially expressed, so candidates obviously can be sought among genes found to be differentially expressed using differential display (24) or new chip technology (1, 11, 50). Of course, differentially expressed candidate loci should map into the defined QTL region. Obviously, if a causative gene is not differentially expressed, it will not be detected in studies directed at differential expression. An excellent example of the use of chip technology to identify a differentially expressed insulin resistance QTL candidate is given by Aitman et al. (1). A major problem in hypertension research is to know which tissue should be examined for differential expression. Kidney and blood vessels are obvious target tissues, but endocrine organs and nervous tissue are also likely tissues for expressing genes influencing BP.

Finally, consideration can be given to sequencing a minimal congenic region. This is hardly a trivial undertaking because 1 CM in the mouse (and presumably in the rat) is approximately equivalent to 2 × 10^6 bases (231). In addition to an ability to do sequencing on this scale, it would be critical to have informatics resources to identify previously known genes or any new genes in the sequence.

Suppose one has a minimal congenic strain that differs in BP from the background strain and in which a candidate locus resides that has functional variants between the congenic and background strain. This is not definitive proof that the candidate is the causative locus because the minimal congenic region is still going to harbor many genes. An average gene (in humans) averages 40–50 kb (60); thus a congenic region of 0.5 cM in the rat might contain on the order of 20 genes. Ultimate proof that a candidate locus is actually a QTL will require targeted allelic substitution where only the putative BP allelic variant from a donor inbred strain replaces the homologous allele in a recipient inbred strain and results in a BP change in the recipient strain. The idea is to be substituting only one gene as opposed to a small congenic region containing many genes.

At this point, mammalian targeted gene substitution followed by creation of an intact animal can be done only in the mouse using embryo stem cells, and it is problematic whether a workable stem cell system can be developed in other mammalian species. One could contemplate putting rat variants into the mouse to study their effect on BP. This is not entirely satisfactory because the effects of the candidate variant would have been defined on a specific inbred rat genetic background that would be confounded in the mouse experiment. Recent advances in mammalian (sheep, cow, mouse) cloning by insertion of somatic cell nuclei into enucleated eggs (21, 107, 220, 262) do provide at least a theoretical way to do targeted gene substitution (possibly in the rat) without using embryo stem cells. Somatic cells from an inbred donor rat strain could be grown in culture and undergo homologous recombination with transfected DNA; the appropriate recombinant cell clones could be selected using techniques presently used on embryo stem cells (220). This could be followed by developing an animal from the nuclei of such recombinant cells. Such a recombinant animal could be bred to the donor strain, and the recombinant chromosome could eventually be fixed in the homozygous state on the genetic background of the inbred donor strain providing the original somatic cell line. The BP of the recombinant strain with the precisely substituted putative BP allelic variant would then be compared with the BP of the donor strain providing the somatic cells.

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