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The Rat as an Experimental Animal

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The development and characterization of many inbred, congenic, and recombinant strains of rats in recent years has led to the detailed genetic description of this species, especially in regard to its major histocompatibility complex. This information has contributed substantially to the study of comparative genetics and has greatly enhanced the utility of the rat in a variety of areas of biomedical research. This article focuses on the use of the rat in immunogenetics, transplantation, cancer-risk assessment, cardiovascular diseases, and behavior.

THE RAT IS A MAJOR EXPERIMENTAL ANIMAL IN TRANSPLANTATION, immunology, genetics, cancer research, pharmacology, physiology, neurosciences, and aging. The strains and randomly bred stocks that have been used almost exclusively are derived from the Norway rat (*Rattus norvegicus*), which is thought to have originated in the area between the Caspian Sea and Tobolsk, extending as far east as Lake Baikal in Siberia. It spread to Europe and the United States with the development of commerce in the 18th century, and by the middle of the 19th century it was being used extensively for studies in anatomy, physiology, and nutrition. The first inbred lines were developed at the beginning of the 20th century by H. H. Donaldson, W. E. Castle, and their colleagues for studies in basic genetics and in cancer research (*1*). Further development and genetic characterization of inbred, congenic, and recombinant strains occurred in the United States, Japan, and Czechoslovakia (*2*), and several reviews have documented these developments in detail (*3–5*). In addition to its experimental uses, the rat has a worldwide economic and medical impact, since it destroys one-fifth of the world's crops each year, carries many diseases that are pathogenic for humans, and kills many children by direct attack (*6*).

This review will focus on current work utilizing the rat in immunogenetics, transplantation, cancer-risk assessment, cardiovascular diseases, and behavior. In these areas of research, the rat has the advantage of being a well-characterized, intermediate-sized rodent without the disadvantages, both scientific and economic, of larger animals and without many of the technical disadvantages of smaller rodents.

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Immunogenetics

Considerable effort has been expended in recent years to develop and characterize inbred, congenic, and recombinant strains of rats, and a wide variety of these genetic resources is now available (*3, 4, 7–9*). Several compilations of basic data have been assembled (*5*), and current developments are regularly updated in the *Workshops on Alloantigenic Systems of the Rat* (*10*) and in the *Rat Newsletter* (*11*). This work has also provided insight into the comparative genetics of the major histocompatibility complex (MHC) and of MHC-linked genes affecting growth and development. The level of polymorphism of MHC antigens in the rat is very low compared to that of other species; the class I antigens have been most extensively studied. Nonetheless, the resistance to disease, reproductive capacity, and ecological stability of the rat do not differ from those of other species. Hence, the biological significance of MHC polymorphism remains a mystery.

The structure of the MHC in the rat (*RT1*) based on data from serological, molecular, and functional studies is shown in Fig. 1 (*3, 12, 13*). The general organization of the class I and class II loci is the same as in the mouse but different from that in all other species studied: the class II loci are interspersed between class I loci rather than following them sequentially (*14*). This observation indicates that (i) the rat and the mouse formed separate genuses after the divergence of the prototypic Muridae, (ii) the evolutionary conservation of the MHC persists despite internal rearrangements, and (iii) the function of these loci does not depend, at least to a first approximation, on their specific order or on their polymorphism.

The *RT1.A* and *RT1.E* loci encode classical class I transplantation antigens and appear to be the homologs of the mouse *H-2K* and *H-2D* loci. There are several other class I loci in the vicinity of *RT1.A*, and the best defined are the diallelic *RT1.F* and *Pa* (pregnancy-associated) loci (*3, 13, 16*). The antigen encoded by the *Pa* locus was first identified on the surface of the basal trophoblast in the allogeneic WF(u) × DA(a) mating by alloantisera and by monoclonal antibodies made by the WF mother (*17*). This antigen carries an epitope that is broadly shared among other class I antigens, but does not have the allele-specific epitope of a classical class I transplantation antigen. Immunohistochemical and electron microscopic studies (*18*) showed that both the Pa and A^a antigens are also on most somatic tissues and that they are carried by separate molecules. The mapping of the *A*, *F*, and *Pa* loci is based on the use of various combinations of inbred, congenic, and recombinant strains; a number of monoclonal antibodies; and specifically designed alloantisera. No recombinants among these loci have yet been found, but immunoprecipitation and peptide mapping studies have demonstrated that they are separate molecules: hence, the order of these loci in Fig. 1 must be considered tentative. The *RT1.G* and *RT1.C* loci encode class I antigens that appear to be homologous to the mouse Qa/TL antigens, but these loci have not yet been well characterized (*19*).

The class II loci *RT1.B* and *RT1.D* were detected serologically and by molecular analysis (*3*), whereas *RT1.H* has been detected only by molecular analysis (*12*). The *B* and *D* loci appear to be homologous to the mouse *A* and *E* loci, and the *H* locus appears to be homologous, in part, to the mouse *ψAβ3* pseudogene and the human *HLA-DP* locus.

The growth and reproduction complex (*grc*) is closely linked to the MHC (*20*). In the homozygous state, it is semilethal in males and females, causes small body weight in both males and females (*dw-3*), and causes male sterility and reduced female fertility (*fi*). These defects are similar to some of those associated with the *t* haplotypes in the mouse, but the *grc* is not homologous to the *t* genes since it does not cause segregation distortion or suppression of recombination (*3, 20*). The fertility defect occurs at the same stage of gametogenesis in both males and females: there is complete arrest of spermatogenesis at the primary spermatocyte stage, and a partial defect in the maturation of the primary ovarian follicle. The *grc* acts at an early stage of meiotic prophase I; it is not associated with any known chromosomal or hormonal abnormality; and it increases susceptibility to chemical carcinogens in both males and females (*21*). Its effects are probably due to the deletion of a segment of the chromosome close to the MHC (*22*). If so, then the increased susceptibility to cancer may be due to the loss of cancer suppressor genes, or anti-oncogenes, as in retinoblastoma and Wilms' tumor in humans (*23*). Hence, these animals may provide a unique system in which to study the genetics of susceptibility to cancer.

The homozygous *grc* genotype (20 to 25% in utero mortality) can interact with the heterozygous *Tal*/+ gene, which is a recessive lethal gene on a different chromosome. The *Tal* gene is not lethal in the heterozygous state but, when homozygous, causes the death of all embryos at 10 to 14 days of gestational age (*24*). This demonstration in mammals of a lethal epistatic interaction, which is the interaction between genes on different chromosomes, provides a useful system in which to study gene interaction during development.

Molecular analysis has delineated the major regions of the rat MHC on the basis of restriction fragment length polymorphisms (RFLPs) (*13, 22, 25*). There are approximately the same number of class I–hybridizing fragments of DNA as in the mouse (*26*), despite the much lower level of serological polymorphism in the rat (*3*). The class II loci have not been examined in any detail yet, but there is a "hotspot" of recombination in the *RT1.H* region.

The biochemical comparisons among the rat, mouse, and human MHC class I and class II antigens are summarized in Table 1. The amino acid sequences of the rat class I and class II antigens are more homologous to those of the mouse than to those of the human, although both levels of homology are fairly high. The homology among antigens encoded by the same class I locus is the same in the rat and the mouse, and both are lower than in the human. The homology between antigens encoded by different class I loci of the same haplotype is much higher in the rat than in the mouse or the human, whereas the interlocus homology for the class II antigens is approximately the same for all three genuses. When one compares the rat with the mouse and the human the most striking difference is in the number of serologically defined class I and class II antigens. This difference has been documented most extensively for the class I antigens in both inbred (*3*) and wild (*27*) populations; it has been less extensively studied for the class II antigens. The class I and class II antigens present in both the inbred and wild populations are serologically and functionally indistinguishable, and there is a high degree of linkage disequilibrium among the loci in the MHC of the rat (*27*).

The difference between the rat and the mouse and human in the serological polymorphism of their class I antigens stands in contrast to the similarity of their RFLP patterns (20 to 36 class I–hybridizing fragments) (*3, 22, 25*). This observation might reflect a similarity in the total number of class I genes in all three genuses but a difference in the number of functional genes. The situation with the class II loci in the rat appears to be the same: their serological polymorphism is very low but their RFLP is high (*3, 12*). Thus, the rat is an extremely useful animal in which to study the control of the functional activity of MHC loci and the biological consequences thereof.

The limited MHC antigen polymorphism in the rat raises the question of what the biological significance of MHC polymorphism is (*28*). Neither the host defense mechanisms nor the reproductive capacity of the rat appear to differ from those of the mouse and the

human, and the rat has certainly prospered in an otherwise hostile environment (*6*). Current thinking assigns a central role to class I antigens in the presentation of foreign antigens to the host immune system and to class II antigens in the recognition of foreign antigens. If these are, indeed, the primary functions of the MHC antigens, then either the specificities of their antigen-recognizing structures are much broader than those of the antibody combining sites or the extent of their antigen-recognition repertoire is not reflected in their serological polymorphism. There is also the relevant, and intriguing, observation that the MHC polymorphism in the protochordate *Botryllus* is the same as that in the mouse and the human (*29*). Why? Only more extensive structural studies of MHC antigens at both the protein and DNA levels will provide the crucial insights into the biological significance of MHC antigen polymorphism.

Transplantation

The rat is the animal most often used in organ transplantation studies: its size makes surgical procedures feasible, provides large amounts of cells and serum, and allows serial biopsies of the transplanted organ to assess the rejection process. The advances in rat immunogenetics over the past two decades have enhanced considerably its usefulness in transplantation research. The rejection times of various organs in different strain combinations have been documented (*5*), and the roles of the different MHC and non-MHC antigens in this process (*30*) have been examined by the use of different combination of inbred, congenic, and recombinant strains. Such transplantation studies have been done with skin (*7, 30*), kidney (*31*), heart (*32*), bone marrow (*33*), liver (*34*), small bowel (*35, 36*), pancreas (*37*), and brain (*38, 39*). There are four major areas of current interest in experimental transplantation research, and the rat is the crucial animal in each of them: allotransplantation of the small bowel, heart, and liver; neural transplantation; xenografting; and reproduction.

Allografting. In systemic allotransplantation, grafting of the small bowel is the most pressing area of study (*35, 36*). Loss of function in this organ occurs in a variety of situations and at all stages of life: for example, congenital abnormalities, necrotizing enterocolitis, mesenteric artery thrombosis, and trauma. The problems encountered include the proper preservation and restoration of the physiological function of this delicate organ. The immunological problems are those of the host-versus-graft reaction by the recipient's immune system and the graft-versus-host reaction by the lymphoid tissue in the Peyer's patches of the graft. In this sense, small bowel grafting presents the same type of tissue matching problems as bone marrow grafting, but the offending T cells cannot be removed from the bowel graft as easily as they can from the bone marrow graft.

Two other important areas of research in allografting are heart grafting and liver grafting. The most critical issue in the long-term survival of cardiac transplant patients is the development of atherosclerosis in the coronary arteries of the transplant (*40*). In humans, this process can lead to the loss of the transplant in 5 to 7 years, so an understanding of its pathogenesis will provide a cogent insight into its therapy. In human liver transplantation, the role of histocompatibility (HLA) matching in the survival of the transplant has not been clarified, and there is the suggestion that under certain circumstances matching can reduce the survival of the graft (*41*). The liver transplantation model has been well developed in the rat (*34*), and it should provide the appropriate system in which to explore these questions.

Neural transplantation. The rat has been an important animal in the study of allogeneic and xenogeneic neural transplantation. Embryonic neural tissue can be transplanted into neonatal and adult brains where it can mature and integrate into the host brain. Both allografts and xenografts can survive for prolonged periods, but they are always susceptible to immune rejection either spontaneously or after challenge by related antigens or by mechanical trauma to the central nervous system (*38*). In the rejection process, however it is precipitated, the host astrocytes are induced to express MHC class I and class II antigens, and the control of such expression may be central to the acceptance of the neural transplant. Cyclosporine A can effectively prolong neural grafts (*42*). Recent studies in humans (*43*) suggest that grafts of neuroectodermal origin can be performed, but such grafts have not yet proven to be clinically useful for any significant period of time. The critical factors that affect the success of a neural transplant are the technique and site of the transplant, the amount of disruption of the blood-brain barrier, the size and source of the donor tissue, the vascularization of the transplant, the age of the host and of the donor at the time of transplantation, and the immunogenetic difference between host and donor.

Studies in rats have shown that such transplants can reduce cognitive defects due to frontal cortex lesions (*44*), improve impairment of motor function in aged animals (*45*), and make functional connections in an allogeneic or xenogeneic setting (*46*). These studies are also providing insight into the immunological status of the brain and the immune reactivity in this organ and into the pathogenesis of focal neurodegenerative diseases (*38*).

The potential value of neural grafts in clinical medicine lies in replacement of damaged neural circuits and in the replacement of cells making chemicals that modulate neural function. Neural circuit replacement might be used to treat trauma in adults and congenital neurological defects in children, and it is in the latter that long-term possibilities for the therapeutic use of neural grafting lie. The use of transplanted cells as a substitute for chemical replacement therapy is complicated by the fact that many of the diseases causing such deficits may have an autoimmune basis, so the transplanted cells themselves may fall victim to the underlying disease process. Much basic work must be done to clarify the immunological and neurophysiological aspects of neural transplantation, the development of specific immunosuppressive regimens for neural transplants, and the pathogenesis of the neurodegenerative diseases for which it might be used as therapy. The effort is worthwhile, since transplantation of tissue into the brain is one of the most promising approaches to have come from experimental neurobiology as potential therapy for a variety of disorders involving damage to the central nervous system. Finally, the use of neural xenotransplants in humans is a distinct possibility (*38*), and the ethical dilemmas raised by this procedure must be examined.

Xenografts. The use of grafts from animals of different families and genuses, xenografting, has been explored sporadically (*47*) and has recently had a resurgence because of the interesting basic immunological questions that it raises and because of the possibility of the use of such grafts as neural transplants (*38*) and as temporary expedients ("bridging grafts") in humans.

Each xenograft system has its own peculiarities (*47*): thus, it is not possible, at the present time, to generalize about the nature of the immune response to xenografts. In order to explore systematically the immunobiology and immunogenetics of xenografting, three areas of resarch should be developed. First, xenoantigens should be identified and characterized. The relative immunogenicity of various xenografts should be studied in one donor-recipient combination in order to develop a coherent body of knowledge that can serve as a paradigm for other systems. The rat-mouse combination will be the most useful one to study initially, because both species are immunologically and genetically well defined. This research should explore (i) the possible existence of unique xenoantigenic systems, (ii) the role of donor MHC antigens in eliciting an immune response to the

xenograft, (iii) the cumulative effect that weak antigenic systems have in xenograft rejection, and (iv) the genesis and nature of "natural" or "preformed" antibodies. As an extension of this line of work, the role that the evolutionary distance between donor and recipient plays in the magnitude of the immune response to the xenograft should be examined. Second, the immune response to the xenograft should be analyzed systematically and in detail, including an investigation of the origin and specificities of preformed antibodies. The latter study may provide some insight into methods for controlling their formation. Third, the mechanism of xenograft rejection should be compared to that of allograft rejection to determine whether the major differences between them are qualitative or quantitative.

Reproductive immunology and genetics. This area has as its central theme the mechanism by which the fetal allograft survives (*48*). The rat is an important experimental animal for examining the nature of the trophoblast antigens and the genetic control of their expression. The allele-specific, class I transplantation antigens are not expressed on the trophoblast surface in allogeneic pregnancies, but they are on the surface in syngeneic pregnancies; in both types of pregnancies, they are present in the cytoplasm (*18*). The Pa antigen is expressed on the trophoblast surface and in the trophoblast cytoplasm in both allogeneic and syngeneic placentas; class II antigens are not expressed in either type of placenta (*18*). This differential antigen expression may be an important factor in the maternal acceptance of the allogeneic placenta. Recent work shows that all of the class I antigens expressed in the placenta are of paternal origin, and this is the first example at the antigen level of genomic imprinting, which is a critical process in reproductive genetics (*49*). The very low level of MHC antigen polymorphism in the rat is crucial to the discrimination needed for these types of studies.

Recessive lethal genes are important causes of fetal death in experimental animals, and they may play an important role in recurrent spontaneous abortion in humans (*48, 50*). The *grc* in the rat, as discussed above, provides a unique model system in which to study these effects. This area of research is an important bridge between the aspects of reproduction of primary interest in the field of transplantation and the broader field of developmental genetics.

Risk Assessment for Potential Carcinogens

The rat has been used frequently for prediction of the effects of chemicals on humans (*51*). For studies of teratogenesis, the advantages of the rat include the ease of counting corpora lutea when assessing the effects of chemicals on ovulation and implantation (*52*), a large litter size, a short gestation period, and a well-studied embryology. However, the susceptibility and sensitivity of rats to particular teratogenic agents may be low when compared with the mouse and the rabbit (*52*), and there are significant differences from man in the effects of chemicals on the fetus (*53*). In mutagenesis studies, the rat appears to offer little inherent advantage over several other species (*54*). It is in the field of carcinogenic risk assessment that the rat has played a prominent role and will continue to do so.

Prediction of carcinogenicity for a given chemical is a major concern for government, the chemical industry, and the public. The development of cancer usually involves, at some stage, an agent or agents foreign to the cell—including xenobiotics, radiation, and oncogenic viruses. Carcinogenesis is a multistep process frequently involving a genotoxic (DNA-altering) step resulting in the alteration of cell division, growth, and differentiation (*55*). Different chemicals, including some with similar structures, may work by different mechanisms, and the cellular differences among tissues further complicate the process. Often one, or sometimes more, specific activated metabolite of a chemical may be the ultimate carcinogen (*56*); hence, different tissues and species of animals may respond differently to any given chemical based on their inherent metabolic patterns. The many unknown aspects of the induction of cancer, the long latency period between exposure and overt disease, and the potential for carcinogenesis at low doses of chemicals have made risk assessment an extremely difficult exercise.

Ultimately, it is epidemiologic studies of humans that will confirm the ability of an agent to cause human cancer (*57*), but such studies are usually performed only after exposure of large populations. This situation has led to the development of carcinogenic risk assessment methodologies that utilize nonhuman test systems (*53*). Assessment of carcinogenicity involves long-term dietary, parenteral, or topical application of the chemical to various mammalian species (*58*). The rat features prominently in such studies because of a favorable combination of small body size, ease of breeding, and relatively low spontaneous tumor rates. The choice of the strain of rats that is used is important in view of the variation in spontaneous tumor rates and different responses to chemicals among inbred strains (*58*). More recently, it has become apparent that such long-term bioassays may occasionally produce conflicting results, as occurred initially with vinylidene chloride (*59, 60*), or may be used with agents such as arsenic that exhibit sufficient evidence of carcinogenicity in humans but limited evidence in animal tests (*60*). Furthermore, because the mechanisms of chemical carcinogenesis have become better understood and the potential for simultaneous exposure to several chemicals has become apparent, chemicals may in the future be assessed for their activity at different stages of the multistep carcinogenic process (*61*).

The long-term application of a test chemical to animals will continue to be the fundamental method of carcinogenic risk assessment because short-term, and particularly in vitro, tests cannot mimic all of the aspects of animal metabolism and physiology (*62*). The long-term bioassays should be done over a large part of the life span of the species, starting in utero, in order to eliminate false negative results due to the prolonged latency of carcinogenic effects. In this respect, the rat is a suitable experimental animal because of its relatively short life span.

In view of the important role played by metabolic enzymes in activating chemicals to reactive carcinogens, the question arises as to whether the rat is metabolically an appropriate substitute for humans. Crouch and Wilson (*63*), using the National Cancer Institute long-term bioassay data and a mathematical formula for carcinogenic potency, demonstrated that the ratio of potency between humans and rats was, on average, within a fivefold range; however, for a given chemical it varied from 1500:1 to 0.02:1. The range of potencies was less divergent between mice and rats, although Bernstein *et al.* (*64*) have argued that this lack of divergence may be a statistical artifact inherent in the long-term bioassays. Purchase (*65*) analyzed 250 chemicals for carcinogenicity in rats and mice based on data from the National Cancer Institute, International Agency for Research on Cancer, and U.S. Public Health Service, and his analysis indicated that a chemical carcinogenic in one species had a 15% chance of not being carcinogenic in the other. These data emphasized the importance of testing chemicals in more than one species in long-term bioassays (*58*). The rat is clearly an appropriate choice for one of these species because so much is known about its metabolic and physiological patterns and because various classes of chemicals are carcinogenic for rats (*53, 59*).

Recent studies on mechanisms of chemical carcinogenesis have demonstrated deficiencies in long-term animal carcinogenesis testing when it is used as the sole assessment criterion, because problems may occur with chemicals that are carcinogenic but that cause only moderate tumor incidence in a given tissue in different

species (*59*). Certain chemicals, notably epigenetic (non–DNA altering) ones, may affect a particular stage of the multistep carcinogenic process initiated by another chemical without being themselves active in a long-term bioassay when tested alone. These facts, together with the increasing costs and slowness of long-term testing, have forced consideration of assays that require less time. Weisburger and Williams (*59*) outlined a decision-point approach to testing whereby chemicals might be analyzed in four increasingly complex classes of carcinogenicity assessment. These classes are as follows: (i) Analysis of the structure of the chemical. This analysis considers the reactivity of the chemical and its metabolites based on structure (*66*). (ii) Short-term tests in vitro. A battery of tests is used including prokaryotic and mammalian mutagenesis systems and studies of direct effects on DNA and chromosomes. (iii) Limited bioassays in vivo. The formation of preneoplastic lesions or rapid tumor induction is assessed in selected species. (iv) Long-term bioassays in vivo. A positive result in these studies is increased overt tumor formation or tumor-induced death of the animal.

For limited bioassay procedures, the induction of breast cancer in female Sprague-Dawley rats and the induction of altered foci in the rat liver may be useful. Cellular and subcellular preparations from rat livers are also commonly used for metabolic activation of chemicals in short-term carcinogenesis and mutagenesis tests (*67, 68*). Coculture of rat hepatocytes with liver epithelial-type cells has been reported to sustain high levels of hepatocyte, carcinogen-metabolizing cytochrome P-450 enzymes (*69*). Such procedures may extend the utility of in vitro hepatocyte cell lines in toxicity testing. The comprehensive assessment proposal of Weisburger and Williams (*59*) is not an established procedure (*58*), but rather illustrates potential future directions for carcinogenic risk assessment. The rat plays an important role in short-term in vitro tests and in limited in vivo bioassays.

The rat has been the most frequently studied species in the in vivo bioassay system of altered liver-focus induction. Research into the cellular events in the course of chemically induced tumor formation has characterized many of the changes that precede malignancy (*70, 71*). Cell populations affected by the carcinogen generally appear as characteristically altered foci detectable by sensitive immunohistochemical reactions, and they appear much earlier than tumor formation. Induction of such foci is not an unequivocal indicator of ultimate malignancy, and their significance in the development of malignancy is debated (*70*). Nevertheless, this assay has been proposed as a limited in vivo bioassay system in carcinogenicity assessment (*59, 70, 72*). Pereira and Stoner (*73*) have reported that the rat liver focus assay exhibited greater sensitivity and fewer false negatives that the strain A mouse lung adenoma assay [some limitations of which are discussed in (*53*)] in detecting genotoxic carcinogens. Parodi *et al.* (*74*) concluded that, at least for a small group of chemicals active predominantly in the liver, assays for liver focus and nodule formation were as accurate, and possibly more accurate, in detecting carcinogenicity than was the Ames test. Preneoplastic lesions have been studied in tissues other than the liver, but a systematic evaluation of their use in bioassays has not been reported (*75*). In view of the large amount of knowledge concerning liver focus formation in the rat (*72*), it is clear that this species will feature prominently in potential bioassay applications. Strains of rats carrying the growth and reproduction complex (*grc*), which is linked to the MHC, exhibit enhanced focus formation compared to wild-type rats when exposed to chemical carcinogens (*21, 76*), and they are candidates for development of highly sensitive liver-focus bioassays.

In the future of carcinogenicity assessment, there is increasing interest in subdividing the carcinogenic process and studying individual stages. As more is learned about the multistep mechanisms, it may be possible to develop assays for the identification of agents that predispose cells to malignancy at specific steps in the process; one such system has already been described for the rat (*61*). With the increasing emphasis on genetic mechanisms in carcinogenesis, the availability of randomly bred, outbred, inbred, and congenic strains of rats (*3–5*) will make this species even more useful in risk assessment as well as in studies on the basic mechanisms of carcinogenesis.

Cardiovascular Diseases

The extensive body of knowledge regarding nutrition, endocrinology, metabolism, and physiology; the detailed studies on anatomy and histology; and the convenient size of the rat make it a particularly useful experimental animal for cardiovascular research. Reproducible, genetically determined abnormalities have been discovered in rat populations that have proven useful in examining the cardiovascular effects of hypertension, obesity, diabetes, and other metabolic diseases (*4, 77*) and a variety of congenital abnormalities of the cardiovascular system (*78*).

Early studies indicated that this species was quite different from humans in its serum lipid and lipoprotein constitution and that it was very difficult to produce sustained hyperlipidemia in the rat (*79*). Until approximately 1950, many attempts to produce atheromatous lesions in the rat had failed in spite of the extensive knowledge about the effects of nutritional manipulation in this species. Then, in the early 1950s simultaneous reports from three laboratories indicated that this resistance could be overcome under the proper experimental conditions (*80–82*). Each study was designed to capitalize on the newly emerging concepts of risk factors for atherosclerosis, and each utilized rats whose resistance to atherogenesis was diminished by unique ways of producing hypercholesterolemia. Hartroft and his colleagues (*80*) and Wissler and his group (*81*) fed rats special diets designed to raise their blood cholesterol levels and then induced hypertension or renal disease or fed the rats chemicals such as propyl thiouracil and sodium cholate. Malinow and his associates (*82*) utilized particularly potent dietary imbalances plus thyroid-depressing agents to induce atherosclerotic lesions. Some of the major findings emerging from these studies were the greater involvement of the coronary arteries than of the aorta, the location of the aortic lesions in the proximal part of the

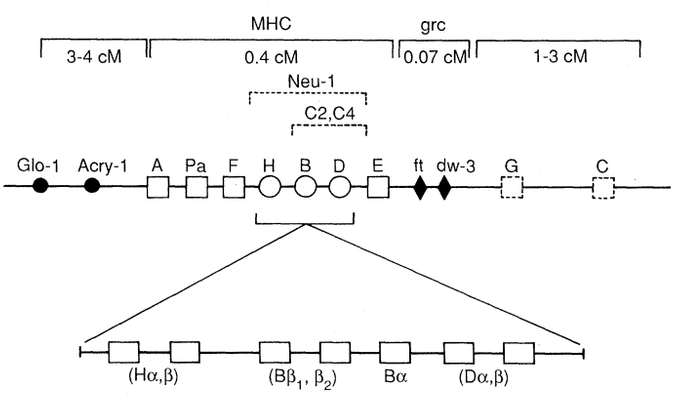

Fig. 1. The major histocompatibility complex of the rat. □, Class I major (classical) transplantation antigens; the dashed squares, the class I medial transplantation antigens; ○, class II antigens; ●, loci controlling polymorphic proteins (Glo-1, glyoxylase I; Acry-1, α-crystallin-1); and ◆, the loci of the *grc* (ft, fertility; dw-3, dwarf-3). The loci indicated by brackets have been mapped to the regions indicated (Neu-1, neuraminidase-1; C, complement components). The evidence for this mapping is presented in (*3, 12, 13*). A cytogenetic study (*15*) has placed the MHC on chromosome 14 of the rat.

Table 1. Amino acid homologies between MHC class I and class II antigens of the rat and those of the mouse and the human (*3, 14, 101*).

Type	Comparison	Percentage homologies					Approximate number of serologically defined alleles*		
		Rat compared to		Allelic and interlocus homologies*					
		Mouse	Human	Rat	Mouse	Human	Rat	Mouse	Human
Class I	Signal peptide	85	50	68–73 (*A*)	32–69 (*K*)	85–95 (*A*)	12 (*A*)	92 (*K*)	24 (*A*)
	α_1 domain	71–73	68		34–57 (*D*)	93 (*B*)	2 (*E*)	63 (*D*)	52 (*B*)
	α_2 domain	71–78	67	97–98 (*A:E*)	36–69 (*K:D*)	79–85 (*A:B*)	4 (*C*)	2 (*L*)	11 (*C*)
	α_3 domain	87	72						
	Transmembrane-cytoplasmic domain	38–46	40						
Class II		80–91	73–81	56–59 (*B:D*)	52–60 (*A:E*)	64–66 (*DR:DQ*)	10 (*B,D*)	74 (*A*) 72 (*E*)	20 (*DR*) 9 (*DQ*) 6 (*DP*)

*Locus or loci compared given in parentheses.

ascending thoracic aorta, and the additive influence of multiple risk factors (*83*). In subsequent studies this model was used to define the influences of various kinds of food fats (*84*) and of metabolic manipulations (*85*) and to delineate the ultrastructural features of these lesions (*86*). In the latter studies, the lesions resemble the foam cell lesions of the rabbit and of other animals in which the blood cholesterol had very high values and in which there was some degree of endothelial injury (*87*). The availability of a wide variety of genetically defined strains of rats will now allow studies such as these to be designed to explore the genetic basis of the various risk factors involved in atherogenesis.

Two inbred strains of rats are particularly useful for studying the pathogenesis of cardiovascular diseases: the SHR (spontaneously hypertensive) strain (*88*) and the BB strain, which spontaneously develops insulin-dependent diabetes mellitus (*89*). The SHR rats develop hypertension that increases with age; is more severe in males; leads to cerebral, myocardial, vascular, and renal lesions; and is responsive to antihypertensive agents. The hypertension is a genetically transmitted trait that is most likely polygenic, and in well-maintained colonies all of the animals develop hypertension between 5 and 10 weeks of age. The inbred, genetically related WKY strain is often used as the normotensive control for the SHR strain. Stroke-prone (*90*) and obese (*91*) substrains of the SHR strain have been developed, but they are difficult to select and maintain because these phenotypic traits most likely have a polygenic basis. The onset of diabetes in the BB rats is rapid, occurs around 90 days of age, affects both males and females, and is under polygenic control, one component of which is linked to the MHC. The clinical syndrome consists of hyperglycemia, hypoinsulinemia, ketosis, polyuria, glycosuria, and weight loss. Pathologic examination shows selective inflammatory destruction of the beta cells of the islets of Langerhans in the pancreas, and the inflammatory process has a substantial immunological component.

Behavior

The rat has been used for studies in behavior since the turn of the century, and a substantial literature has emerged from these studies (*92, 93*). The investigation of the hereditary and environmental aspects of learning began with the introduction of maze experiments by Small (*94*) and led to the development of "maze-bright" and "maze-dull" lines of rats by selective breeding (*95*). Various emotional characteristics have been developed in rats by selective breeding (*93, 96*), and the role of different areas of the brain in behavior has been investigated by stimulation and by extirpation experiments (*44, 45, 97*). Finally, the effects of aging (*93, 98*) and of various pharmacological agents, including alcohol (*99*) and narcotics (*100*), on behavior have been explored.

These studies have provided insights into behavior and into its anatomic and physiologic basis and have led to the development of the field of experimental psychology. However, the lines of rats used were not developed according to the standard rules of genetic inbreeding, and they generally led, at best, to populations with a restricted genetic composition, relative to a randomly breeding population of rats, in which a certain phenotypic characteristic was prominent. This situation has complicated the more detailed genetic interpretation of much of the experimental literature on behavior, and it is particularly acute when examining the relative roles of heredity and environment in learning. One possible approach to developing appropriate strains of rats for behavioral studies may be to select partially inbred rats for their behavioral characteristics and then to breed them for these traits in the context of a mating scheme that would also continue the inbreeding.

Concluding Remarks

The rat is a major experimental animal in all fields of biomedical research and technology, and studies with it have provided much basic and applied knowledge. Its greatest utility has been in those fields broadly classified as experimental pathology and experimental surgery. The extensive work done on the immunology and genetics of the rat in recent decades has greatly enhanced its utility and has contributed substantially to the body of knowledge in immunogenetics. As the constraints on the use of larger animals grow, the rat should provide an excellent alternative to their use. Such a change would also have the advantage of allowing more sophisticated studies to be designed, since so much is known about the biology of the rat, and this would greatly enhance the value of the experiments done.

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Research Articles

Defining the Inside and Outside of a Catalytic RNA Molecule

JOHN A. LATHAM AND THOMAS R. CECH

Ribozymes are RNA molecules that catalyze biochemical reactions. Fe(II)-EDTA, a solvent-based reagent which cleaves both double- and single-stranded RNA, was used to investigate the structure of the *Tetrahymena* ribozyme. Regions of cleavage alternate with regions of substantial protection along the entire RNA molecule. In particular, most of the catalytic core shows greatly reduced cleavage. These data constitute experimental evidence that an RNA enzyme, like a protein enzyme, has an interior and an exterior. Determination of positions where the phosphodiester backbone of the RNA is on the inside or on the outside of the molecule provides major constraints for modeling the three-dimensional structure of the *Tetrahymena* ribozyme. This approach should be generally informative for structured RNA molecules.

RNA CATALYSIS, OBSERVED INITIALLY IN THE SELF-SPLICING of the precursor to the large ribosomal RNA (rRNA) of *Tetrahymena*, is not an isolated phenomenon (*1*). RNA self-splicing has been identified as a property of a number of other introns found in precursor RNA from both prokaryotes and eukaryotes, and ribonuclease P has been established as an enzyme with a catalytic subunit composed of RNA (*2*). In all cases, the structure of the RNA itself must form the catalytic center to perform precise RNA cleavage-ligation or hydrolysis reactions.

Recent work on a shortened form of the *Tetrahymena* intron, the L−21 Sca I RNA, has shown that this RNA is capable of mediating a variety of transesterification reactions (*3, 4*). The L−21 Sca I RNA has saturable binding sites for substrates and performs transesterification in a multiple turnover format identical to that of a classical enzyme.

There is, however, a striking difference between the L−21 Sca I RNA and classical protein enzymes. Proteins are assembled from amino acids whose side chains include both hydrophobic and hydrophilic functional groups. Nonpolar amino acids are usually in the interior of the catalyst where hydrophobic interactions are maximized, while the charged and polar amino acids are concentrated on the exterior and thus maximize interaction with the solvent. Catalytic RNA, like other RNA, consists of the four nucleotides. These do not have the structural diversity of amino acid side chains. The phosphates are anionic, and additional hydrophilicity comes from the sugar and base functional groups.

Like proteins, these ribozymes (RNA enzymes) require a specific structure for their biochemical activity. RNA secondary structure has been proposed by comparative sequence analysis of related group I introns, and confirmed by analysis of splicing defective mutations and second site suppressors that restore activity (*5, 6*). A

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