

GROWTH AND REPRODUCTION COMPLEX IN THE RAT  
Genes Linked to the Major Histocompatibility Complex  
That Affect Development\*

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The growth and reproduction complex (Grc)<sup>1</sup> of the rat is a group of genes linked to the major histocompatibility complex (MHC) which influence body size and fertility (1). Both males and females are small, the males are sterile because of an arrest of spermatogenesis at the early pachytene state of the primary spermatocytes, and the females have a reduced reproductive capacity. The Grc can be separated from the MHC by recombination, and the genes influencing body size (dw-3) and reproductive capacity (ft)<sup>2</sup> can also be separated by recombination. There is no apparent sex influence on the inheritance of the Grc, at least as it is presently understood, because it can be transmitted by either males or females. This unique developmental defect does not appear to have either a chromosomal (2) or hormonal (1, 3) basis. The Grc is of intrinsic biological interest in the study of the genetic regulation of reproduction and development, and it provides an easily detectable phenotypic marker for use in linkage studies involving the loci of the MHC.

This study was undertaken for several reasons. First, it is necessary to explore in detail the effects of the Grc on growth and reproduction in a cross involving an animal with good reproductive characteristics. Second, the cross must contain a large enough number of animals so that a significant number of recombinants can be obtained to get the accurate map distance between the MHC and the Grc. Third, further evidence that the genes in the Grc can be separated into those influencing growth (dw-3) and those influencing reproduction (ft) and an accurate map distance between them must be obtained. Finally, it is desirable to establish a line in which there is a recombination between the genes of the MHC and the Grc to explore further the relationships between these two constellations of genes.

### Materials and Methods

Segregation of the Grc was examined in F<sub>2</sub> hybrids derived from BIL/1 females (RT1<sup>h</sup>) and YO (RT1<sup>u</sup>) males. The animals of the YO strain (YO 38366) are very large, in contrast to the

\* Supported by grant HD 08662 from the National Institutes of Health and by the Beaver County Cancer Society and the Tim Caracio Memorial Cancer Fund.

<sup>1</sup> *Abbreviations used in this paper:* DHT, 5- $\alpha$ -dihydrotestosterone; FSH, follicle-stimulating hormone; Grc, growth and reproduction complex; LH, luteinizing hormone; MHC, major histocompatibility complex.

<sup>2</sup> The gene symbol for the locus that affects fertility was formerly called f (1), but it has been called to the authors' attention that this symbol had already been used for the fawn mutation. Hence, the symbol ft will now be used for the gene(s) in the Grc which affects fertility.

small size of the BIL/1 strain, and they have excellent reproductive capabilities. This cross yielded 1,722 animals, of which 1,568 reached weaning and were studied. The animals were fed rat chow and water *ad libitum* and were kept in a conventional colony with the lights on for 12 h. They were weighed and examined three times a week on the same day relative to their birth using a Mettler P-1200 (Mettler Instrument Corp., Hightstown, N. J.) or Torbal PL-1 (Torsion Balance Co., Clifton, N. J.) balance. This schedule permitted convenient data management and statistical analysis. The animals were typed for their RT1.A antigens by the hemagglutination technique as described previously (4).

The phenotypic criteria for normal animals were their RT1 haplotype, normal body weight, and normal fertility; normal adult males had testes weighing  $\sim 1.5$  g. Two classes of animals generally fell into this category: RT1<sup>u</sup> homozygotes, which did not carry the Grc, and RT1<sup>1/u</sup> animals, which were heterozygous for the Grc. The phenotypic criteria for animals homozygous for the Grc were a body weight  $< 2$  SD from the mean of their normal littermates and homozygosity for the RT1<sup>1</sup> haplotype. The females had reduced fertility, and the males were sterile as a result of aspermatogenesis, as confirmed by histology, and had testes  $\sim 1/10$  the size of normal (0.15 g). In the studies on neonates, the animals were typed using cardiac blood within 3 d of birth.

The testes or ovaries were removed at autopsy, trimmed, fixed in 10% formalin for 10 d, dried, and weighed on a Mettler H-20 balance. The animal's weight at the time of autopsy was used to calculate the gonad:body weight ratio. The gonads were sectioned and examined microscopically with hematoxylin and eosin and Feulgen staining. Morphometric studies were done with an ocular micrometer.

In the studies on the number of ova/ovary released during an estrous cycle, the animals were killed at the time of ovulation and the number of corpora lutea in each ovary was counted in step sections stained with hematoxylin and eosin. The time of ovulation was determined by vaginal smearing. Fetal mortality was determined by counting the number of fetuses in each uterine horn at day 8 of gestation by laparotomy under halothane anesthesia and in the same horn at day 20. The animals were killed at day 20 and typed for their RT1 haplotypes. The time of conception was determined by vaginal smearing.

The differences among the growth curves for animals of the various haplotypes were determined by an analysis of variance of repeated measures (5) using data only from animals for which all weights from age 30 to 130 d in 10-d increments were available. There were significant variations in growth among animals carrying various haplotypes within each sex and between males and females with the same haplotype. The methods for calculating the recombination frequency and its 95% confidence limits in an F<sub>2</sub> cross utilize the Punnett square and the binomial distribution, respectively.

## Results

The body and testicular weights of the (BIL/1  $\times$  YO)F<sub>2</sub> hybrids segregated with the RT1 haplotype (Table I), and the relative weight differences remained constant throughout life (Fig. 1): the RT1<sup>1</sup> homozygotes were significantly smaller than the RT1<sup>u</sup> homozygotes and the RT1<sup>1/u</sup> heterozygotes. The growth rates (g body weight/d) of all males were constant from 30 to 70 d of age (Table II). Those of the RT1<sup>u/u</sup>, RT1<sup>1/u</sup>, and YO animals were essentially the same, and those of the RT1<sup>1/1</sup> and BIL/1 animals were slower. The growth rates of all females were constant from 30 to 60 d of age, and the same patterns of growth were seen (Table II). In both males and females, the BIL/1 strain appeared to have a slightly slower growth rate than the RT1<sup>1/1</sup> animals.

The testicular weights of the RT1<sup>1</sup> homozygotes relative to body weights were the same as those of the BIL/1 strain and considerably less than in the RT1<sup>1/u</sup> and RT1<sup>u/u</sup> animals (Fig. 2). The variance in relative testicular weights was the same in the latter two types of animals. Histologically, there was no sperm production by the testes of the RT1<sup>1</sup> homozygotes because of an arrest of spermatogenesis at the early pachytene

TABLE I  
Average Body and Gonad Weights of Adult Males and Females (3-5 mo of age)

Animals	Male			Female		
	Number	Body weight (g)	Testis weight (g)	Number	Body weight (g)	Ovary weight (g)
	<i>mean ± SD</i>			<i>mean ± SD</i>		
F <sub>2</sub> hybrid						
(l/l)	31	273 ± 32	0.190 ± 0.030	29	166 ± 21	0.021 ± 0.005
(l/u)	89	403 ± 36	1.578 ± 0.207	70	230 ± 23	0.029 ± 0.005
(u/u)	49	410 ± 28	1.584 ± 0.138	56	230 ± 19	0.031 ± 0.006
YO strain	15	393 ± 110	1.103 ± 0.308	21	224 ± 20	0.027 ± 0.006
BIL/1 strain	5	204 ± 24	0.154 ± 0.020	5	134 ± 10	0.015 ± 0.004

stage of the primary spermatocyte. The testes of the RT1<sup>u</sup> homozygotes were larger than those of the YO strain, and this finding may be a result of the effects of hybrid vigor. There were no differences in ovarian weights among the three types of F<sub>2</sub> hybrids, the parent BIL/1 strain and the YO strain (Table I and Fig. 2), but the ovaries of the RT1<sup>l</sup> homozygous females, which had reduced fertility, produced fewer ova/estrous cycle (Table III).

The litter sizes, sex ratios, and RT1 haplotypes of the (BIL/1 × YO)F<sub>2</sub> hybrid offspring at birth and at weaning are shown in Table IV. There was distortion of the RT1 phenotypic ratios in the male and female offspring because of a lack of RT1<sup>l</sup> homozygous animals, i.e., those homozygous for the Grc. If the litters were divided into those in which there was preweaning death and those in which there was no preweaning death, the same distortion of phenotypic ratios occurred in the former group only. In the litters with no preweaning death, there was no significant phenotypic distortion when the males and females were examined individually, but there was some distortion in the total population. The average size at birth of the litters without preweaning death was smaller than that of the litters with preweaning death, but it was the same at weaning in both populations.

To examine this question further and to obtain some insight into where the loss of the RT1<sup>l</sup> homozygotes occurred, the RT1 haplotypes of neonates and the fetal loss between days 8 and 20 of gestation were examined. The average litter size of the neonates (Table IV) was intermediate between those of the litters with and without preweaning death, and it showed the same pattern as the litters with no preweaning death. There was no significant phenotypic distortion in the males, and the distortion in the females and in the total population was only marginal. There was no significant difference between the number of fetuses at day 8 and day 20 of gestation in animals that carried the Grc and in those without it (Table V). These data indicate that the loss of animals carrying the Grc occurred in the immediate postnatal period.

In the 1,568 weaned animals studied, 8 recombinants were found, and their characteristics are shown in Table VI. Seven of the recombinants were between the MHC, as determined by the RT1.A antigen, and the Grc. One animal had a recombination between the genes influencing body size (dw-3) and fertility (ft). Because this animal had the RT1<sup>u</sup> haplotype and a normal body size, but small infertile testes, the recombination occurred between the MHC and DW-3 (wild type

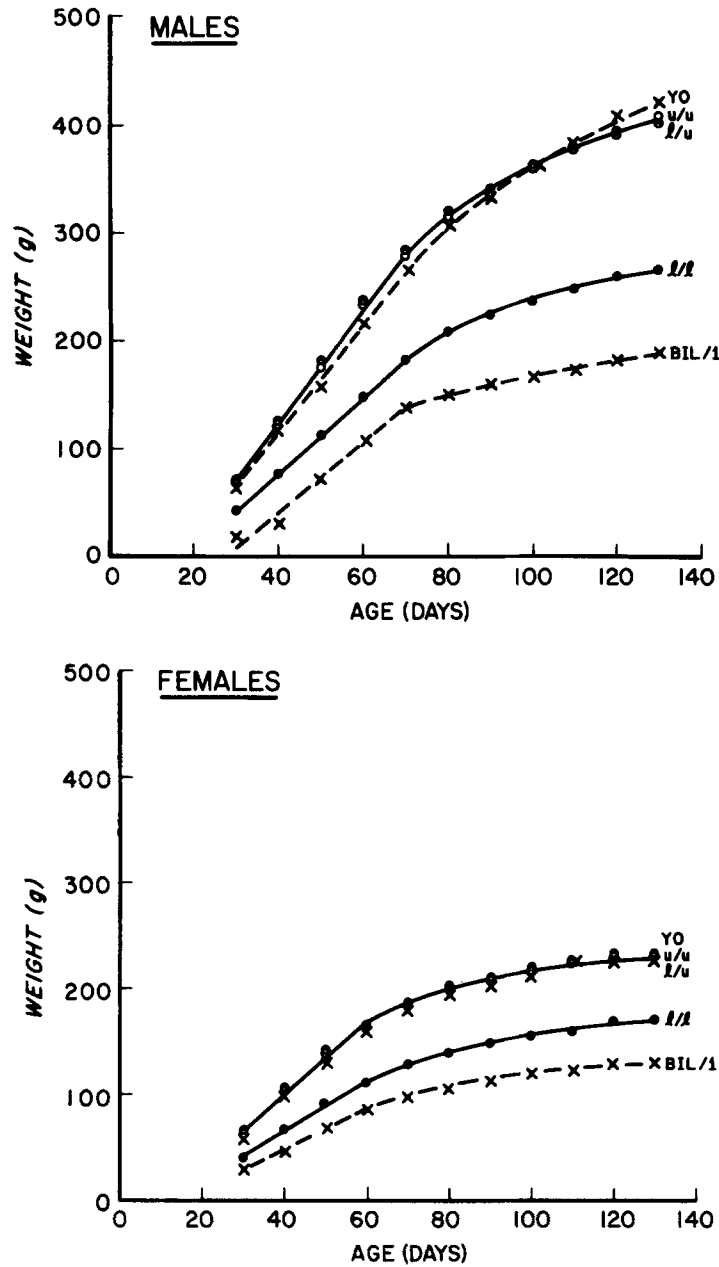


FIG. 1. The growth curves of the (BIL/1 × YO)F<sub>2</sub> hybrids (haplotypes l/l, l/u, and u/u) and the parent YO and BIL/1 strains. The curves were drawn with data obtained from animals that had weights at each one of the time points relative to birth. The growth curves were linear from days 30-70 for the male and days 30-60 for the female. The average standard deviation, calculated from the variance of all the data, was 10.56% of the body weight. The number of animals used for each curve was (female, male): l/l, 27, 25; l/u, 67, 85; u/u, 51, 46; YO, 21, 14, and BIL/1, 5, 5.

TABLE II  
Growth Rate of the  $F_2$  Hybrids and the Parental Strains Obtained from the Slopes of the Linear Portions of the Growth Curves

Sex	Number of animals	$F_2$ haplotype or strain	Growth rate	
			Mean	95% confidence limits
				<i>g/d</i>
Male*	78	u/u	5.3	4.7-5.9
	187	l/u	5.2	4.8-5.7
	18	YO	4.8	3.9-5.8
	56	l/l	3.4	3.0-3.9
	5	BIL/1	3.2	§
Female‡	149	u/u	3.3	2.6-4.0
	272	l/u	3.1	2.8-3.3
	24	YO	3.2	2.4-4.1
	97	l/l	2.4	2.2-2.6
	5	BIL/1	2.3	§

\* Linear growth from days 30 to 70.

‡ Linear growth from days 30 to 60.

§ There were not enough animals to obtain a reliable estimate of the 95% confidence limits.

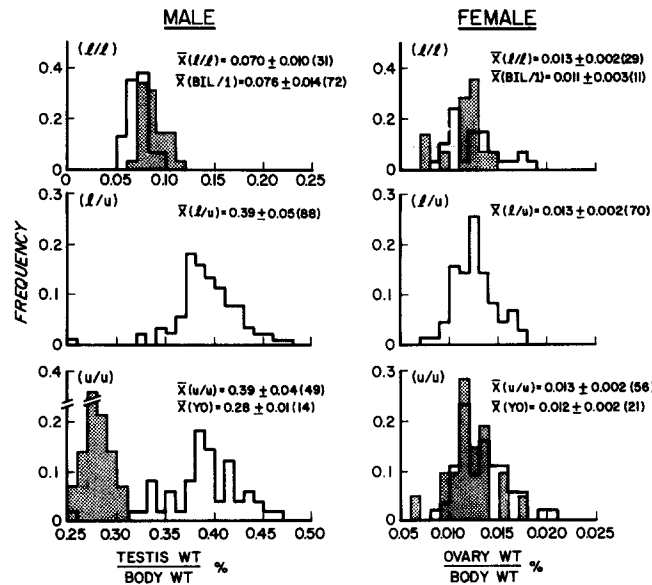


FIG. 2. Distribution of the testicular and ovarian weights as a percentage of body weight for the three haplotypes of the (BIL/1 × YO) $F_2$  hybrid and the parental YO and BIL/1 strains. The shaded areas are the data for the parental strains. The average percentage, standard deviation, and number of animals used for the calculations are shown on the graph. The frequency interval for calculations of the testis/body weight was 0.01%, and for the ovary/body weight, 0.001%.

at the locus influencing body size) on the one hand and the gene(s) influencing fertility (ft) on the other.

The evidence supporting recombination in the four male recombinants (Table VI)



TABLE V  
Fetal Loss between Days 8 and 20 of Gestation in Animals Carrying the *Grc*

Mating	Chromosomes (RT1.A-Grc)	No.	Day 8	Day 20	Day 20 compared to day 8	RT1 haplotype of fetuses at day 20		
						l/l	l/n	n/n
BY1* × BIL	$\begin{array}{cc} \overbrace{1 \quad \text{Grc}} & \times & \overbrace{1 \quad \text{Grc}} \\ \underbrace{1 \quad \text{Grc}} & & \underbrace{n \quad +} \end{array}$	6	6.83 ± 2.64	4.17 ± 2.79	>0.05	9	15	
BIL × BIL	$\begin{array}{cc} \overbrace{1 \quad \text{Grc}} & \times & \overbrace{1 \quad \text{Grc}} \\ \underbrace{n \quad +} & & \underbrace{n \quad +} \end{array}$	15	9.00 ± 1.81	7.73 ± 1.87	>0.10	27	49 32	
BI × BI	$\begin{array}{cc} \overbrace{n \quad +} & \times & \overbrace{n \quad +} \\ \underbrace{n \quad +} & & \underbrace{n \quad +} \end{array}$	5	8.80 ± 2.95	6.80 ± 3.03	>0.05	34		

\* BY1 is the RT1<sup>1</sup> homozygote of the (BIL/1 × YO)F<sub>2</sub> hybrid offspring.

‡ One-tailed Mann-Whitney U test.

TABLE VI  
Recombinants Found in 1568 (BIL/1 × YO)F<sub>2</sub> Hybrid Offspring

No.	Recombinants			Body weight and size			Gonad weight, size, and function			Type of recombination	
	Sex	RT1 haplotype	Age	Weight	Size	Normal range*	Weight‡	Percentage of body weight§	Size		Presence of sperm or ova
117	M	l/u	4	268	S¶	392-429	0.176	0.058	S	0	MHC/Grc
401	M	l/u	4	248	S	404-435	0.162	0.060	S	0	MHC/Grc
1,573	M	l/u	1.8	67	S	147-194	0.130	0.054	S	0	MHC/Grc
206	F	l/l	3.5	247	N	240-260	0.139	0.020	N	+	MHC/Grc
671	F	l/l	2.5	225	N	191-200	0.065	0.016	N	+	MHC/Grc
342	F	l/l	3	249	N	193-215	0.047	0.010	N	+	MHC/Grc
1,684	F	l/l	2	150**	N	162-187	0.039	0.029		+	MHC/Grc
976	M	u/u	2	156‡‡	N	135-163	0.029	0.110	S	0	MHC-DW-3/ft

\* From normal littermates.

‡ Normal value for a testis is 1.584 ± 0.138 (SD) g (49 animals), and for an ovary, 0.031 ± 0.006 g (56 animals) (Table I).

§ Normal value for a testis is 0.39 ± 0.05%, and for an ovary, 0.013 ± 0.002% (Fig. 2). The weights at autopsy (4-6 mo) were used for this calculation: 291, 248, 250, 247, 301, 340, ND, and 302 g for recombinants 117, 401, 1,573, 206, 671, 342, 1,684, and 976, respectively.

|| 0, absent; +, present. The testicular defect is described in detail elsewhere (1).

¶ S, small; N, normal; and ND, not done.

\*\* A small (l/l) littermate weighed 111 g.

‡‡ A small (l/l) littermate weighed 97 g.

were progeny tested with males of the BIL-(RT1<sup>1/n</sup>) strain, which is heterozygous for the *Grc*. The recombinant females should carry one chromosome bearing the RT1 haplotype and the *Grc*, and another chromosome carrying RT1<sup>1</sup> but wild for the *Grc*.

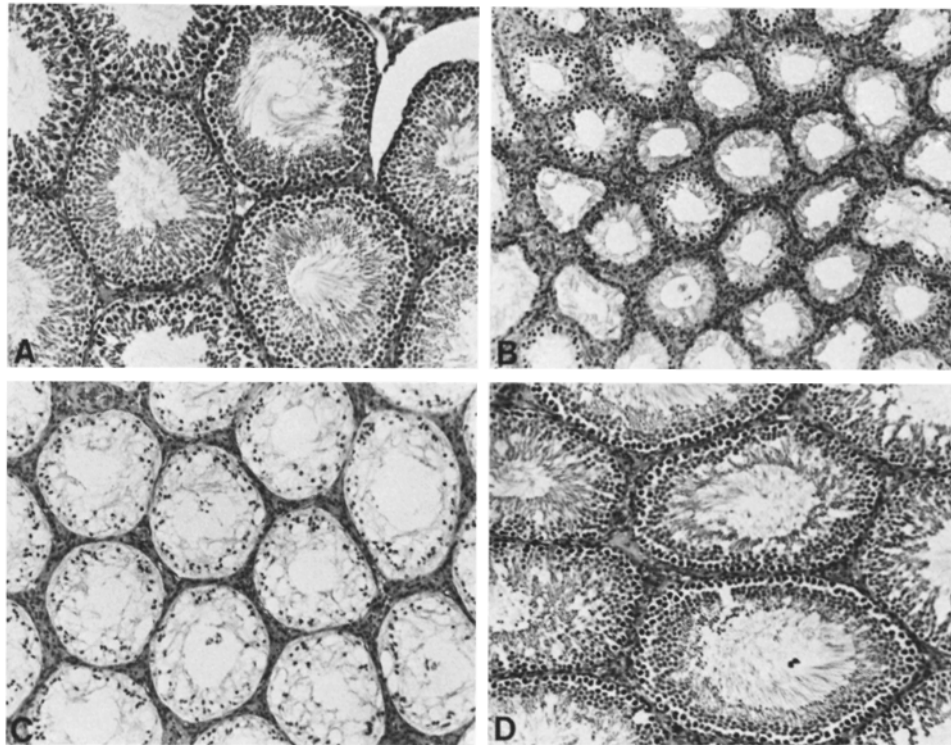


FIG. 3. The histological appearance of the testicular tubules from various animals. (A) The tubules of the BI strain. There is a well-developed and well-differentiated germinal epithelium, and each of the seminiferous tubules contains a large number of sperm. (B) The tubules of recombinant 117. There is an arrest of the germinal epithelium at the early pachytene stage of the primary spermatocytes, and the tubules are small. This picture is characteristic of all of the recombinants between the MHC and Grc. (C) The tubules from recombinant 976, in which the recombination took place between DW-3 and ft. The tubules show an arrest of spermatogenesis at the spermatogonia stage, and there are only a few germinal cells present in each tubule. The predominant cell type is a vacuolated Sertoli cell. However, the tubules are significantly larger than those in the recombinants between the MHC and Grc, but still smaller than normal. (D) The seminiferous tubules of male 4048, which was used to generate a line in which there was a recombination between the MHC (l/l) and the Grc (wild type) (Fig. 4). This animal has completely normal seminiferous tubules. Hematoxylin and eosin staining.  $\times 150$ .

The data in Table VIII show that this was the case for each of them: the offspring that were homozygous for  $RT1^l$  divided into those with normal and small weights, and all of the  $RT1^{l/n}$  animals were normal. The 7/1568 MHC/Grc recombinants give a map distance between  $RT1.A$  and  $dw-3$  of 0.45 (0.25–0.96) centimorgans, and the 1/1568 recombinant between  $DW-3$  and  $ft$  gives a map distance of 0.07 (0.04–0.40) centimorgans.

The female recombinant number 206 was used to start an inbred line for future studies on the nature of the region between the MHC and the Grc. The recombinant chromosome carried the  $RT1^l$  haplotype and was wild for the Grc ( $RT1.A^l-Grc^+$ ): the objective of the mating scheme was to isolate this recombinant chromosome in an inbred line (Fig. 4). The recombinant was mated with a BIL male, and approximately two-thirds of the  $RT1^l$  homozygous offspring had normal body weights, as expected. All of the  $RT1^{l/n}$  offspring were normal and they were not used further. That the



TABLE VII  
*Size and Sperm Production of Testicular Tubules in the BI Strain and in the Various Recombinants*

Animal	Size of testicular tubules ( $\mu\text{m}$ )*		Comparison to BI‡	Sperm production§
	Major axis	Minor axis		
	<i>mean <math>\pm</math> SD</i>			
BI	316 $\pm$ 43	251 $\pm$ 33		++++
117	145 $\pm$ 10	132 $\pm$ 12	—	0
976	226¶ $\pm$ 26	173¶ $\pm$ 6	—	0
4048**	338 $\pm$ 46	265 $\pm$ 26	0	++++

\* Average of 10 tubules. The shape of the tubule was best approximated as an ellipse.

‡ One-tailed Mann-Whitney U test. —, decreased; and 0, no change.  $P \leq 0.01$ .

§ Arbitrary scale of 0 to +++++. In those animals with sperm, the sperm was present in all tubules examined. The estimates were made on hematoxylin-and-eosin- and on Feulgen-stained sections at 200 X.

|| Recombinants 401 and 1573 were essentially the same.

¶ Larger ( $P < 0.001$ ) than in recombinant 117.

\*\* See Fig. 4.

TABLE VIII  
*Test Mating of Recombinant Female Progeny With Males of the BIL Strain*

Recombinant number*	Offspring of the test mating with BIL males ( $\text{RT1}^{l/n}$ )					
	Body size			Body size		
	RT1 haplo-type	Normal	Small	RT1 haplo-type	Normal	Small
206	l/l	11	6	l/n	18	0
671	l/l	9	3	l/n	7	0
342	l/l	8	5	l/n	3	0
1684	l/l	3	4	l/n	12	0

\* All recombinants were  $\text{RT1}^{l/l}$  and had normal body weights (Table VI).

normal-sized  $\text{RT1}^{l/l}$  animals were heterozygous for the Grc was established both by mating a small animal, which is homozygous for the Grc, with a normal-size animal and by mating two normal-size animals. In all three crosses, there were both normal and small animals. A variety of offspring of female 2039 and male 2044 were tested with either male BIL animals or with female BIL/1 animals to find those offspring which carried the recombinant chromosome, i.e., those that did not give any small progeny in this test mating. Female 4038 and male 4048 were such animals, and they were selected to start the inbred line. After mating, male 4048 was examined histologically and it had normal-size testicular tubules (Table VII) with a normal germinal epithelium (Fig. 3). This mating pair produced only normal offspring, and this finding provides a second level of proof that they carried only the recombinant ( $\text{RT1.A}^1\text{-Grc}^+$ ) chromosome. The line was propagated by brother  $\times$  sister mating, and all animals in any one generation will be selected for mating in such a way that they trace back to a common pair of ancestors within five to seven generations. It will be designated as the BIL(R1) strain.

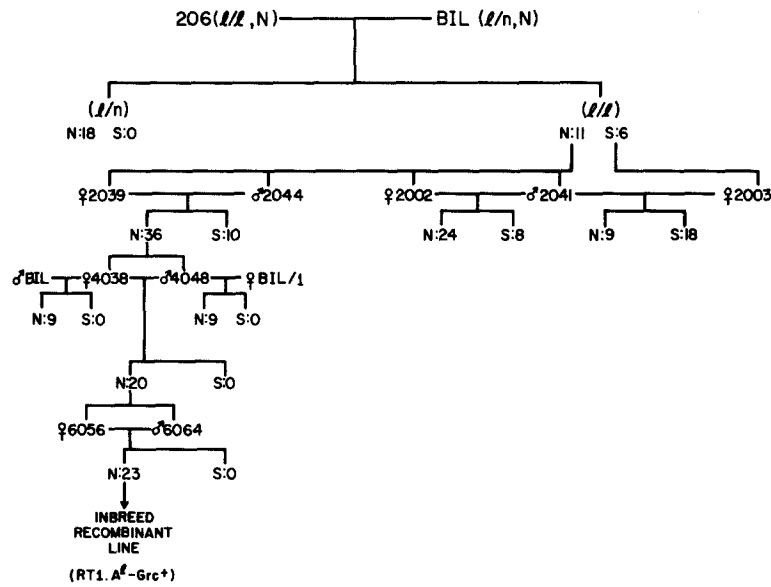


FIG. 4. The pedigree for the development of a recombinant line in which the recombination occurred between the RT1<sup>l</sup> haplotype of the MHC and the wild-type genes of the Grc (RT1<sup>l</sup>-Grc<sup>+</sup>). Female recombinant 206 was mated with a BIL male, and the (1/1) offspring were test-mated to see which ones carried only chromosomes wild for the Grc. Two of these animals, female 4038 and male 4048, were selected to develop the recombinant line, which is currently in the F5 generation. That they were wild for the Grc was confirmed by test mating with the BIL or BIL/1 strain and by histology (Fig. 3).

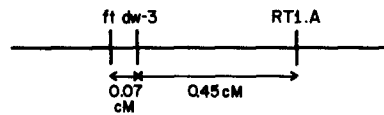


FIG. 5. The map distances between the RT1.A locus of the MHC and the dw-3 and ft loci of the Grc. The relative orientation of dw-3 and ft is based on one recombinant, so it must be considered tentative. cM, centimorgans.

### Discussion

These studies establish the map distance between the MHC and the Grc and the division of the Grc into two genes or gene complexes, one of which influences small body size (dw-3), and the other influences male sterility and reduced female fertility (ft). The orientation and map distances of these three loci are shown in Fig. 5. The orientation of the loci is based on the finding that recombinant 976 had small infertile testes but a normal body weight and the RT1<sup>u</sup> haplotype. The latter haplotype is associated with a normal body weight, so the most probable interpretation of the recombination is that it occurred between a segment of chromosome carrying RT1.A and DW-3 and a segment carrying ft. There is the theoretical possibility that this animal might be a double recombinant, which would change the gene order, but given the map distances involved, this event is highly improbable. The separation of the effects ascribed to dw-3 and ft has previously been observed, and this finding reinforces the conclusion presented here that there are at least two genes, or gene clusters, within the Grc and that each influences separate developmental processes. As

noted below, however, the two genes may interact in the expression of their primary functions.

Studies to explore the potential mechanism of action of the genes in the Grc provided strong evidence that it did not depend upon chromosomal abnormalities (2) or endocrinological abnormalities (1, 3). Morphological studies on metaphase chromosomes of BIL rats by conventional Giemsa-trypsin staining, and comparison of the Giemsa-banded karyotypes of the rats that carried the defects with the karyotypes of other strains did not reveal any gross chromosomal abnormalities. Extensive endocrinological study of the RT1<sup>1</sup> homozygotes that carried the Grc and their normal RT1<sup>u</sup> littermates from the F<sub>2</sub> hybrid population revealed no defects that could explain the lack of spermatogenesis. First, the following functions were the same in both the RT1<sup>u</sup> and RT1<sup>1</sup> homozygotes: conversion of 5  $\alpha$ -dihydrotestosterone (DHT) to 3  $\alpha$ -androstenediol; DHT localization in the testes; luteinizing hormone (LH) and follicle-stimulating hormone (FSH) production in castrates in response to systemic testosterone; testosterone production in response to LH stimulation; serum testosterone, growth hormone, and somatomedin concentrations; and testicular temperature. Even though basal LH and FSH levels were slightly higher in the RT1<sup>1</sup> homozygotes, their postcastration responses to the hormones were normal. Second, the RT1<sup>1</sup> homozygotes did not show any sperm production in response to an intratesticular testosterone implant or in response to systemic FSH administration. Third, tissue homogenates from the testes of the RT1<sup>u</sup> and RT1<sup>1</sup> homozygotes showed the same binding constant for LH ( $2.4 \times 10^{10} \text{ M}^{-1}$ ) and the same competitive inhibition of the binding of radioiodinated FSH by unlabeled FSH in a radioreceptor assay. Both homozygotes had approximately the same number of LH binding sites per interstitial cell (5,000–10,000). Finally, the histological structure of the prostate, seminal vesicles, and preputial gland in the RT1<sup>1</sup> homozygotes was normal. However, the testes of the RT1<sup>1</sup> homozygotes showed an arrest of spermatogenesis at the early pachytene stage of the primary spermatocytes.<sup>3</sup>

The gene(s) influencing fertility (ft) appears to act in the testes by preventing the development of the primary spermatocyte (Fig. 3) and in the ovary by decreasing the number of ova produced (Table III). The reduction in the number of ova shed is not a result of the smaller size of the BIL/1 females, because animals of comparable size, e.g., PVG, F344, and OKA, have the largest litter sizes seen in inbred rats (9). There appears to be some interaction between dw-3 and ft because the recombinant between DW-3 and ft (number 976) had significantly larger testes (Table VI) and testicular tubules (Table VII) than the animals carrying both dw-3 and ft. The same size differences were seen in the recombinant between DW-3 and ft reported previously (see Table X, animal 5516, of reference [1]). That FT/ft may be the primary determinant of testicular development is suggested by the previous findings that in a dw-3/FT recombinant the testicular size and sperm production were normal (see Table X, animal 4998, of reference [1]). These three findings provide further evidence for the effects on body size (dw-3) and on gonadal development and function (ft) being a result of separate, but interacting, genes.

<sup>3</sup> The testicular defect associated with the Grc bears some resemblance to that in the LEW/Orl strain of rats, which carries the hypodactyly mutation hd (7, 8). The hd-bearing animal has small, infertile testes in which the tubular epithelium is either devoid of germinal cells and has only Sertoli cells, or there are some germinal cells arrested at the primary spermatocyte stage. This defect is, however, not linked to the MHC (H. W. Kunz and T. J. Gill III. Unpublished data.).

The phenotypic distortion of the RT1 haplotypes in the F<sub>2</sub> hybrid (Table IV) is not a result of intrauterine fetal loss (Table V) but of death in the immediate postnatal period (Table IV). The distortion observed in the entire population was a result of the losses in those litters in which preweaning death occurred. In the litters with no preweaning death and in neonates examined within 3 d of birth, there was only a marginal loss of RT1<sup>l</sup> homozygotes.

The genes of the Grc, particularly the one(s) affecting fertility (ft), bear some relationship to the t-recessive genes of the T/t complex, particularly the semilethal ones. In both conditions, no chromosomal or hormonal abnormalities have been demonstrated. The ft gene in the homozygous state acts at an early stage of meiotic prophase I as does the t<sup>sl</sup> gene (10). Testicular weights in homozygous animals carrying the ft or t<sup>sl</sup> genes are reduced considerably, and the lack of sperm production leads to sterility (11). The effects of both the ft and t<sup>sl</sup> genes are different from those of the recessive lethals, t<sup>l</sup>, in that the latter allow the production of some abnormal sperm and act between sperm production and fertilization (10, 11). The phenotypic distortion of haplotypes in the offspring of rats that carry the Grc cannot be ascribed clearly to a variable transmission ratio, which depends upon a segregation distorter such that described in *Drosophila* (12) and in the mouse (13, 14), even though there is some variation from a strict 2:1 ratio of the l/u heterozygotes to the u/u homozygotes in the F<sub>2</sub> hybrids described here (Table IV) and those described previously (1) and from the 1:1 ratio in backcrosses (1). These differences are relatively small and not of the magnitude generally seen with the variable transmission ratios of the t-recessive alleles in the mouse. Nonetheless, there are striking similarities between the genes of the Grc in the rat and the t<sup>sl</sup> genes of the T/t complex in the mouse. Whether the details of this comparison correlate exactly or not, the essential fact is that there is a gene complex linked to the MHC both in the rat and in the mouse that influences development. This finding suggests that a chromosomal region that affects development and that is linked to the MHC may exist as a general phenomenon in mammals and that the whole chromosomal region might function as a supergene (15).

### Summary

The linkage of the major histocompatibility complex (MHC) and the growth and reproduction complex (Grc) in the rat was studied in an F<sub>2</sub> hybrid population generated from female BIL/1 (RT1<sup>l</sup>-Grc) and male YO (RT1<sup>u</sup>-Grc<sup>+</sup>) animals: 1,722 offspring were born, and 1,568 were weaned and studied. The body weights of the offspring segregated with the RT1 haplotype of the MHC, and the RT1<sup>l</sup> homozygotes were significantly smaller than their RT1<sup>l/u</sup> and RT1<sup>u/u</sup> littermates. The growth rate of the RT1<sup>l/l</sup> animals was approximately the same as that of the BIL/1 animals, and both were significantly less than the growth rates of the RT1<sup>l/u</sup>, RT1<sup>u/u</sup>, and YO (RT1<sup>u</sup>) animals. The testes of the RT1<sup>l</sup> animals showed an arrest of spermatogenesis at the early pachytene stage of the primary spermatocytes, and they were ~1/10 as heavy as the testes of the RT1<sup>l/u</sup> and RT1<sup>u/u</sup> animals. The ovaries in females of all three haplotypes had the same weight, but there was a decrease in the number of ova released per cycle in the RT1<sup>l/l</sup> animals. The major loss of the RT1<sup>l</sup> homozygotes, which caused distortion of the phenotypic ratios among the offspring, did not occur *in utero* but in the early postnatal period before weaning. There were 7/1568 recombinants between the MHC, using the RT1.A antigen as the marker, and the Grc,

using small body size (dw-3) as the marker, and 1/1568 recombinant between the loci influencing body size (dw-3) and fertility (ft) of the Grc. These data gave the following map distances (95% confidence levels): RT1.A to dw-3, 0.45 (0.25–0.96) centimorgans and dw-3 to ft, 0.07 (0.04–0.40) centimorgans. A female recombinant was used to develop an inbred line carrying the RT1.A<sup>1</sup>-Grc<sup>+</sup> chromosome.

We thank Miss Etta Volk and Mrs. Dorothy Johns for their expert technical assistance and Miss Ronda Bauer for her help with the breeding studies.

*Received for publication 23 July 1980.*

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