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The emerging role for rat models in gene discovery

Melinda R. Dwinell^{1,3}, Jozef Lazar^{2,3}, and Aron M. Geurts^{1,3}

¹Medical College of Wisconsin, Departments of Physiology, 8701 Watertown Plank Road, Milwaukee, WI 53226

²Medical College of Wisconsin, Departments of Dermatology, 8701 Watertown Plank Road, Milwaukee, WI 53226

³Medical College of Wisconsin, Departments of Human & Molecular Genetics Center, 8701 Watertown Plank Road, Milwaukee, WI 53226

Abstract

Rat models have been used for many decades to study physiological and pathophysiological mechanisms. Prior to the release of the rat genome and new technologies for targeting gene manipulation, the rat had been the underdog in the genomics era, despite the abundance of physiological data compared to the mouse. The overarching goal of biomedical research is to improve health and advance medical science. Translating human disease gene discovery and validation in the rat, through the use of emerging technologies and integrated tools and databases, is providing power to understand the genetics, environmental influences, and biology of disease. In this review, we will briefly outline the rat models, bioinformatic tools, and technologies that are changing the landscape of translational research. The strategies used to translate disease traits to genes to function, and ultimately, to improve human health will be discussed. Finally, our perspectives on how rat models will continue to positively impact biomedical research will be provided.

Introduction

The traditional role for the laboratory rat (*Rattus norvegicus*) has been to study biological traits and disease mechanisms with the goals of understanding both the normal pathways and identifying gene variants that lead to disease. The rat has long been used for laboratory studies, primarily due to its size, behavior, biology, and cost to maintain in comparison to larger mammals, including primates, and continues to be the preferred animal model for safety and toxicology testing (Kunert et al. 2006; Serikawa et al. 2009). All major organ systems have been extensively studied in the rat, making it an ideal model for systems biology and translational studies. Interestingly, the prevalence of the rat in biomedical research literature is second only to humans (Jacob 2010). The rat genome was released in 2004 (Gibbs et al. 2004), greatly facilitating comparative genomics and cross-species data integration and paving the way for targeted gene manipulation. Although mouse models have had a clear advantage for experimental genetics due to the availability of embryonic stem cells, rat models have been widely used for translational research due to a wealth of phenotypic data and its physiological similarities to humans (Huang et al. 2011).

A survey of PubMed content demonstrates the dominance of the laboratory rat as a research model (Fig. 1). While the mouse is king in some research areas such as immunology and cancers, for most organs and physiological and metabolic systems, the rat is historically

time consuming and expensive process if mapping of traits to the genome begins with two inbred strains.

Chromosomal substitution strains (consomic) were derived by introgressing a single chromosome from one inbred strain (donor strain) to the background of another inbred strain (recipient strain) (Roman et al. 2002). From these consomic strains, congenic strains can be rapidly generated (Cowley et al. 2004) (Fig. 2, left panel) and have a greater statistical power to detect linkage over traditional F2 crosses (Shao et al. 2010). The feasibility of this approach has been demonstrated by an mapping cardiovascular (Cowley et al. 2001), renal disease (Mattson et al. 2008; Schulz et al. 2010), vascular function (Kunert et al. 2006; Kunert et al. 2010), pulmonary hypertension (Bonnet et al. 2006), respiratory control traits (Dwinell et al. 2005), metabolic syndrome (Gilibert et al. 2008), and tumor susceptibility (Adamovic et al. 2008; Adamovic et al. 2010) in the consomic panels derived from the SS (Dahl salt sensitive) and BN (Brown Norway) strains. For example, hypertension QTLs in these models have recently been reduced to single centimorgan sized regions (Joe et al. 2009; Moreno et al. 2011b).

Other strategies have been successfully used to map complex disease traits, such as hypertension and metabolic disorders, to the genome include recombinant inbred strains and heterogeneous stock rats. Recombinant inbred (RI) strains are panels of strains that are derived by inbreeding multiple independent lines from an F2 population established from a cross of two inbred strains. The F2 rats are brother-sister mated for more than 20 generations to ensure a fixed genome. The BXH/HXB RI strains were derived from crosses of SHR/Ola and BN-*Lx*/Cub to produce 20 HXB and BXH RI strains each (Pravenec et al. 1989). These strains have been used to map traits associated with hypertension and metabolic syndrome in a wide variety of tissues and, in combination with gene expression analysis, have identified several candidate genes (Pravenec and Kurtz 2010). Since each RI strain is inbred, follow-up studies can be done on genetically identical animals.

Alternatively, the heterogeneous stock (HS) strains were derived from eight genetically and phenotypically diverse inbred founder strains and then bred for more than 40 generations using a strategy to minimize inbreeding to produce a colony in which each rat is a unique genetic mosaic of the founders (Solberg Woods et al. 2010a). The advantage to this strategy is that the design is similar to human population studies with many possible alleles at any given loci compared to inbred and RI strains with only two alleles at each loci. In this strategy, since each individual animal is genetically unique, follow-up studies on genetically identical rats is not possible, but the great strength of this approach is the ability for rapid fine-mapping of traits since the recombination interval is approximately 2 cM (Mott et al. 2000). With the recent advances in genome technologies, including high-throughput sequencing and high-density SNP chips, further genetic analysis on these strains can be done. This approach has been used successfully for narrowing regions for glucose regulation (Solberg Woods et al. 2010a), bone fragility (Alam et al. 2011), alcohol drinking (Bice et al. 2010), fear (Johannesson et al. 2009), and renal traits (Solberg Woods et al. 2010b).

In some cases, these and other strategies have been used to identify genes for certain phenotypes, although positional cloning has been more successful for monogenic traits (Aitman et al. 2008). The stream of genes associated with complex traits has significantly increased in the past few years due to the combination of new technologies for gene disruption (Petretto et al. 2008; Pravenec et al. 2008a; Pravenec et al. 2008b; Rangel-Filho et al. 2005), advances in sequencing and mapping, and new computational tools available for the rat. In a supreme demonstration, this strategy of using inbred rat models to identify and model specific genes and pathways which can then be translated to humans is highlighted by the landmark papers describing functional Adducin 1 gene variants causing hypertension in

both in rats and in humans and the progress of Rostafuroxin in phase 2 clinical trials (Ferrandi et al. 2010; Lanzani et al. 2010).

Bioinformatic Tools and Data

Concomitant with developing animal resources, developing bioinformatic tools and cataloging data sets are needed to keep up with the volume and variety of data, including strains, genome sequence, including whole genome, exome, and transcriptome, tissue specific (e.g. expression), and physiological data. RGD incorporates information on the more than 650 inbred rat strains, including substrains of the original inbred strains, and also an additional 2275 rat models that have been used to study the genetics of complex disease. These models include consomic, congenic, recombinant inbred, conplastic, transgenic, and strains with targeted mutations. A full listing of all strains used to study disease or map biological traits to the genome are available on the RGD FTP site for download (<ftp://rgd.mcw.edu/pub/>). In total, RGD has curated more than 1850 QTLs for 103 traits mapped to various rat chromosomes by these mapping strategies over a broad range of confidence intervals. Through partnerships with NCBI/Entrez Gene, IPI (International Protein Index), Ensemble, UCSC genome browser, Gene Ontology Consortium, Gene Ontology Annotations (GOA) at European Bioinformatics Institute (EBI), and the Mouse Genome Database (MGD), RGD provides access to curated integrated sequence, gene, mapping, and strain datasets acquired through user submission, manual curation from literature, automated pipelines from other scientific databases, and through local bioinformatic analyses (Dwinell et al. 2009). In addition to current rat data, homology data between rat, mouse and human is captured and updated weekly through automated ortholog relationship and nomenclature pipelines. Finally, RGD data is obtained and displayed by other global genomic resources such as NCBI Ensemble and UCSC to facilitate the entire research community.

The ability to link an animal model by gene, physiological trait, and quantitative trait locus (QTL) offers the prospect for building more accurate animal models of human disease (Fig. 3). With the rapid increase in SNP-trait associations published in GWAS, it has also become clear that many associations are common, associated with modest effect sizes, and often within noncoding regions (Hindorff et al. 2009). Correlations between rat and human QTLs is an important step in building animal disease models. Another important function of RGD is to develop the many tools for querying genes, genomic features, sequence, ontology annotations, protein, and comparative data across rat, mouse and human and have been reviewed recently (Twigger et al. 2008; Worthey et al. 2010). RGD's genome browser (GBrowse, http://http://rgd.mcw.edu/fgb2/gbrowse/rgd_904/) is undergoing an update to specifically allow seamless movement between human and rat GBrowse, making cross-species comparisons of critical genomic elements possible (Fig. 4). The update includes the ability to add tracks for objects such as QTLs, SNPs, strains, and gene models. The user can begin browsing for a gene, QTL, or region for rat or human and then layer on corresponding data from human or rat through the identification of syntenic blocks and objects linked to those blocks. A similar approach can be used to compare SNPs and linkage disequilibrium blocks from human studies to mapped rat QTL regions. These types of comparisons can narrow regions of interest significantly, reducing the number of genes to prioritize for functional follow up studies.

Identifying Candidate Disease Genes

To test candidate genes mapped by crosses in rats or to validate candidate genes found in human studies, a common approach is to disrupt gene function and measure the impact on specific phenotypes. Most recently, methods for specific gene targeting have changed the way rat models can and are being used for these types of studies, and provide the basis for

crucial studies to study the role of candidate-disease causing genes contributing to normal biological processes, how specific variants alter these normal processes, and how these disrupted processes result in disease. Traditional transgenic approaches by DNA microinjection of rat embryos have been established for more than 20 years, resulting in thousands of published studies (Jacob et al. 2010), however, for specifically disrupting gene function, many attempts to derive useful embryonic stem cells from rat strains have failed. It wasn't until late 2008 that independent reports by Qilong Ying and his mentor, Austin Smith, illuminated the path to germline competent embryonic stem cells (gcESCs) from the rat using specific small molecule inhibitors to suppress key differentiation triggers (Buehr et al. 2008; Li et al. 2008). With some coaxing, these cells have now been demonstrated to be amenable to genetic manipulation by homologous gene targeting (Tong et al. 2010) and others have followed and perhaps improved on the inhibitor media formulations to increase the number of gcESC lines from various genetic backgrounds (Kawamata and Ochiya 2010). Theoretically, induced pluripotent stem cells (iPSCs) from different strains could serve as a platform for genetic manipulation such as gene knockout, knockin and conditional mutagenesis if they were proven to be germline competent. To date, we are aware of three groups deriving rat iPSCs using a variety of approaches (Chang et al. 2010; Li et al. 2009; Liao et al. 2009), all by combining genetic reprogramming with variations of the aforementioned inhibitor media cocktails. These reported cell lines share many pluripotent attributes with iPSCs and ESCs from mice and humans such as the ability to differentiate in to tissues from the three germ layers, although none have yet reported germline transmission as has been shown for mouse.

ENU (N-ethyl-N-nitrosourea) mutagenesis has been successfully used by several groups to target genes for the development of specific genetic models (van Bostel et al. 2008; Mashimo et al. 2008; Zan et al. 2003). Specific examples of the utility of this method include models for febrile seizures (Mashimo et al. 2010a), a novel adenomatous polyposis coli (APC) model (Yoshimi et al. 2009), and a 5-HT transporter (SERT) knockout model (Olivier et al. 2008). This strategy has been extended by the Kyoto University Mutant Rat Archive (KURMA) to develop a resource of cryopreserved sperm from thousands of first generation offspring of ENU-mutagenized males. DNA from these offspring can be screened prior to intra-cytoplasmic sperm injection to produce a rat, thus making this strategy more efficient (Mashimo et al. 2008). Although ENU mutagenesis is often used for phenotype-driven approaches, the use of this strategy for a reverse genetics approaches has resulted in rat knockout models and an archive for producing thousands of models in the future.

We and others have recently developed an alternative and rapid approach using Zinc Finger Nucleases (ZFNs) which have the ability to target and disrupt genes directly in the rat embryo (Geurts et al. 2009; Mashimo et al. 2010b; Moreno et al. 2011a). ZFNs are engineered proteins which can be customized to interact with an investigator-specified sequence, such as the coding sequence of a gene of interest, and introduce a mutation. Delivered by standard pronuclear microinjection procedures as ZFN encoding messenger RNA, these molecules have a single purpose: to find their target sequence and introduce a double strand break in the target chromosome sequence. Innate DNA repair mechanisms activate to repair these breaks and, in the process, a mutation is often introduced (Geurts and Moreno 2010). More recently, it has been shown that by co-introducing a targeting plasmid vector with homologous sequences, one can stimulate homologous gene targeting to knock in sequences directly in both the mouse and rat embryo (Cui et al. 2011; Meyer et al. 2010). This technology, therefore, can be applied for both gene knockout and knockin directly in the embryo. The major advantages of this approach are speed and the ability to apply ZFNs to any rat strain which is amenable to obtaining embryos for microinjection. From gene selection to knockout animal can take less than 4 months and we have now applied this

approach to many genes across inbred, outbred, consomic and congenic strains covering seven genetic backgrounds (Table 1). The ability to apply gene disruption technology at this pace across many disease models strains has not been possible in the past, even in mouse, because of limitations in which strains useful gcESCs have been derived.

We have already begun capitalizing on the ZFN knockout approach to address the significant challenge created by the identification of hundreds of genetic variants for many disease traits through GWAS. The PhysGen Knockout team (<http://rgd.mcgw.edu/wg/physgenknockouts>) at the Medical College of Wisconsin has been targeting GWAS genes using the ZFN technology to develop sensitized rat models with targeted mutations for studies aimed at testing the physiological function and the role of these genes in disease progression (Fig. 2, right panel). This program is on track to hit 100 genes in 2 years and phenotype at least 20 of these strains for cardiovascular, renal and vascular traits, demonstrating the speed and efficiency of this approach. Adding the phenotypic characterization of these strains to the existing foundation of rat physiological data will also be of great value.

Translation from Human to Rat and Back Again

Translational research is about transforming progress in basic research into products and procedures that benefit patients. Basic research, using both patients and experimental models, is the only method to accelerate personalized medicine. Progress in translational research is limited by inadequate understanding of the processes during the development and progression of disease. The use of animal models in the study of human complex traits is irreplaceable. A worldwide effort, both in funding and infrastructure, is advancing the use of the rat in comparative genomics, bioinformatics tools, sequencing, model development, and development rat embryonic stem cells (Aitman et al. 2008), all in combination with detailed physiological profiling to provide a powerful translational tool.

The tremendous progress of sequencing technologies and the advent of GWAS had led to an explosion of the number of genetic variants associated with complex diseases. However, progress to understand mechanisms of diseases has been slow. Thus far, there have only been a few loci where the association signal includes a nonsynonymous single nucleotide polymorphism (SNP) triggering a functional characterization. Moreover, the majority of sequence variants have been found in intergenic sequences and some of identified loci reside close to genes whose functions are still unknown. Additionally, the small affect size indicates that others factors such as multiple rare and low-penetrance variations and interacting environmental factors are involved in final phenotype which compromises the translation from signal to function.

Different approaches are available to translate findings in human GWAS and sequencing to animal models and *vice versa*. One example used recombinant inbred strains to study gene networks and their regulatory loci. These data were integrated with human gene expression and GWAS data to identify genes, networks and pathways for human disease (Heinig et al. 2010). Another successful approach has been to use comparative genomics to identify genes most likely to be responsible for regulating plasma lipid levels in humans by integrating GWAS candidate loci, human cis-expression QTL and conserved co-expression modules between human, mouse and rat (Wang et al. 2009). Comparative genomics is also well suited to further resolve a GWA locus and identify the best candidate gene(s). Comparison of human GWA loci with concordant QTLs identified in mouse and rat may facilitate detection of functionally conserved genes and regulatory region (Moreno et al. 2008). Recently, a pediatric patient benefited from whole exome sequencing, followed by an analysis and diagnose that relied upon comparative genomics, to detect the presence of

functional SNPs which lead to a diagnosis and treatment (Mayer et al. 2011; Worthey et al. 2011). In the process of analyzing the whole exome sequence in this young child, a bioinformatics tool was developed to validate the sequence capture, type of variant, predict impact through algorithms, and utilize database annotations to provide functional information related to the SNPs to assist the physicians in diagnosis (Worthey et al. 2011). Current adaptation of this tool for use with rat strain sequence will be of great value in the use of disease models for discovery and translation.

On the Horizon

The use of rat models for biomedical research is growing due to the availability of genetic technologies and the wealth of existing phenotype data. Now that these technologies are in place nearly 20 years after the first knockout mouse was published (Fig. 1b, arrows), they promise to enable many new cellular and genetic approaches in the most widely studied model system. The rapid growth of sequence data for both humans and model organisms is changing the strategy used to identify and validate candidate disease genes. The rat Brown Norway reference sequence is currently being updated, but the most exciting prospect is the availability of other inbred strain sequences. Several strains have already been sequenced and are currently being assembled and annotated to allow investigators to query across commonly used strains (Abbott 2009, 2010; Pravenec and Kurtz 2010). Other new technologies, such as RNAseq for transcriptome sequencing, allow for full genomic characterization of strains. With these additional types of data, strain-specific characterization could include quantification of transcript levels, integration with genomic sequence to assess allelic specific expression, and could also be used to detect strain specific splice variants. Significant challenges exist with the onslaught of strain sequencing, including streamlined methods to detect variants, validate the results, provide annotation, and informatics tools for analysis. The ongoing improvements in rat resources for functional studies will speed future translational efforts and rate of discovery of risk alleles from genome-scale resequencing efforts.

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Fig. 1.

Results from the NCBI PubMed biomedical literature database. **1a:** Total number of references by disease (left panel) or organ system (right panel) in mice (open bars) and rats (solid bars); **1b:** Left panel: Total publications for rats (solid symbols) and mice (open symbols) by year (1980-2010) with arrows indicate when knockout technologies became available for each species; Right panel: comparison of number of publication between mouse and rat using “genes” and “animals, genetically modified” as search criteria.

Fig. 2. Diagram outlining some of the strategies used to identify candidate genes for disease traits. Left panel depicts the more traditional approach of using F2 crosses for QTL identification or animal breeding strategies to narrow the genomic region containing candidate genes. Right panel illustrates a strategy to use targeted mutation in a rat model to validate GWAS SNPs.

Fig. 3. Diagram highlighting the approaches available for use of animal models for translational biology with the need for bioinformatic tools to integrate gene and phenotype data.

Fig. 4. The Rat Genome Database (RGD) genome browser (GBrowse) allows users to view overlap between human and rat QTLs through synteny. **4a:** Browser with Human genome build 36.3 on chromosome 17 with blood pressure QTL shown in red and blue solid lines (first track). The human syntenic blocks are mapped in aqua blue (second track). Corresponding rat syntenic blocks are mapped in yellow (third track). **4b:** Detailed information about the corresponding syntenic block in rat can be obtained by mousing over the syntenic block of interest. **4c:** View of the overlapping rat blood pressure QTLs for the region on human chromosome 17 shown in 4a.

TABLE 1

Summary of genes targeted using the Zinc Finger Nuclease strategy as part of the PhysGen Knockout program (<http://rgd.mcw.edu/wg/physgenknockouts>). The stage of development for each strain is indicated by 1) injected with ZFN (I), 2) backcrossing or intercrossing (B), or 3) completed (C).

GENE	Stage of development	GENE	Stage of development	GENE	Stage of development
Acad10	C	Gm7	I	Pparg	B
Ace	I	Gucyl1a3	C	Prex1	B
Add2	I	Hexim2	C	Prokr1	C
Adipoq	B	Il1rl1	B	Prune	B
Adra2a	B	Itga9	C	Ptpn11	B
Agtr1a	C	Kenj11	B	Rab38	C
Agtr1b	I	Kenj16	I	Rag1	C
Agtrap	C	Kenq1	C	Rasgrp3	C
Aldh2	C	Ldlr	C	Ren	C
Alms1	C	Lep	B	Resp18	I
Apoe	C	Lpin1	B	Sh2b3	C
Asip	C	Lss	I	Slc30a8	C
Atp2b1	B	Mas1	C	Slc34a1	I
Bcas3	C	Mra	C	Slc6a12	B
Beat1	I	Mstn	C	Slc7a9	B
Cacna1h	B	Mthfr	C	Smoc2	I
Cd247	B	Myadml2	B	Sorcs1	C
Cdh13	C	Myliip	B	Sorcs2	B
Clen6	B	Nap5	C	Sorcs3	C
Comt	B	Nat8	B	Stc1	B
Cst3	B	Nos3	I	Stk39	C
Cubn	B	Nox4	C	Tcf7l2	C
Cyp11a1	C	Nppa	C	Tfdp2	B
Cyp4a3	C	Nppb	C	Trafd1	B
Ets1	C	Nr2f2	B	Ube2q2	B

GENE	Stage of development	GENE	Stage of development	GENE	Stage of development
Fgf1	B	Pdc	B	Ulk3	C
Fgf5	B	Plcd3	C	Ulk4	C
Fyn	B	Plekha7	C	Umod	B
Gnb3	B	Plod1	C	Wdr72	C
Gpr183	C				