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Modeling Disease in the Mouse: Lessons From DNA Damage Response and Cell Cycle Control Genes

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Abstract The advent of gene targeting has allowed the dissection of many essential cellular pathways, including those involved in cell cycle regulation, signal transduction, and development. However, it is becoming increasingly clear that the simple gene deletion strategy may not be sufficient for the modeling of many cancer syndromes. In this Prospect article, we will discuss the strengths and weaknesses of mouse models, how they have advanced from gene deletions to truncations, point mutations, and conditional mouse models in which expression or loss of the gene of interest is controlled either temporally or spatially. We will also consider future directions for the use of mouse models in cancer. The vastness of the field necessitates focusing on a few specific examples with the unfortunate exclusion of many excellent studies from our discussion. As such, we focus on a few specific models of human cancer syndromes, however many of the themes discussed here are applicable to other systems of genetic manipulation and may be applied across fields. J. Cell. Biochem. 97: 459–473, 2006. © 2005 Wiley-Liss, Inc.

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Targeted gene disruption in vivo has directly resulted in enormous advances in biomedical research, and the mouse has become a valuable tool to generate controlled genetic changes in order to model and understand human diseases. As such, knockout mice have been invaluable in the advancement of our understanding of tumor biology, yet many knockout mouse models do not accurately recapitulate human cancer syndromes. Indeed, loss of a gene during embryogenesis represents an extremely rare situation in human development, with germline mutations representing only 1% of the lesions leading to tumorigenesis [Fearon, 1997]. In particular, loss of both alleles in the germline (and, therefore, in all cells) is extremely uncommon, as sporadic tumors generally arise through the progressive accumulation of mutations that ultimately give rise to tumors. This caveat imposes certain limitations to the system and requires one to cautiously draw parallels between systems containing total gene loss and human diseases. Thus to mimic the human condition more accurately, deletions, truncations, point mutations, and conditional gene alterations are now frequently being generated. In this review, we discuss what we have learned from various mouse models, including strengths and weaknesses of this powerful system.

WHY MODEL HUMAN DISEASES IN THE MOUSE?

The study and understanding of human disease, in particular cancer, is complicated by many factors—genetic, social, and environmental. Thus the ability to make specific changes in an organism in a controlled environment, and to study the phenotype and understand the basic biological pathways that are affected by such changes, has been instrumental in the understanding of many diseases. Mice have become the system of choice in many laboratories as they provide a genetically tractable system that shares similar genes and organ systems to humans. Mice also breed rapidly, have a relatively short lifespan (approx. 2 years) and significantly, develop tumors making them

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ideal for the study of genes involved in cancer progression. However, there are significant differences between mice and humans that may affect the observed phenotype following genetic manipulation (for an excellent review see [Rangajaran A. Nat Rev Cancer, 2003]).

One example of this is the telomere, which protects the ends of chromosomes by acting as a 'cap' for the DNA ends and preventing them from being recognized as DNA breaks [de Lange, 2002]. Telomeres become shorter upon every cell division due to the inability to completely replicate the lagging strand. Once telomeres become critically short, cells reach replicative senescence, thus telomere length is a critical factor in the lifespan of all eukaryotic cells. Inbred mouse strains have significantly longer telomeres than humans, possibly due to the expression of telomerase, an enzyme that extends telomeres. Mice express telomerase in essentially all cells (i.e., stem and somatic cells), whereas it is not found in somatic human cells. Telomere shortening is overcome in almost all cancerous cells through the activation of telomerase or through alternative mechanisms, and appears to be an essential step in tumorigenesis. Thus, in humans, it appears that short telomeres provide an additional hurdle to uncontrolled growth that is not present in the mouse (for a comprehensive discussion see [Wright and Shay, 2000]). Evidence for this can be seen in mice heterozygous for the tumor suppressor gene, *p53*. $p53^{+/-}$ mice develop a vastly different tumor spectrums depending on whether they have short ($p53^{+/-}$ $mTERC^{-/-}$) or long telomeres ($p53^{+/-}$ $mTERC^{+/+}$) [Artandi et al., 2000]. Significantly, the tumors in $p53^{+/-}$ $mTERC^{-/-}$ mice show complex cytogenetic profiles similar to human carcinomas.

Despite these fundamental differences between species, mouse models have provided invaluable insight into many disease mechanisms and will undoubtedly continue to do so. Models are becoming increasingly advanced and are able to more accurately mimic human disease. Our purpose here is to consider the strengths, as well as the shortcomings, of murine models.

MOUSE MODELS OF HUMAN CANCER SYNDROMES

Essential Gene Knockouts

Nijmegen breakage syndrome (NBS) is an autosomal recessive disorder characterized by microcephaly, growth retardation, immunodeficiency, and increased cancer risk (Table I). Cells from NBS patients exhibit sensitivity to ionizing radiation (IR), increased levels of spontaneous and radiation-induced chromosomal aberrations, T-cell translocations, and a failure to suppress DNA synthesis upon damage (reviewed in [Group, 2000; Digweed and Sperling, 2004]). Nbs1, encoded by the *NBS1* gene and mutated in NBS, is found in a complex with the evolutionarily conserved Mre11 and Rad50 proteins, which have been implicated in sensing damage and double strand break repair (Fig. 1, for review see [Petrini and Stracker, 2003; Stracker et al., 2004]).

Ataxia-telangiectasia (A-T) is a human autosomal recessive disorder characterized by neurodegeneration resulting in ataxia, the loss of motor coordination; and telangiectasia, dilation of small blood vessels in capillary beds that often leads to hyperpermeability and hemorrhage. A-T patients are also immunodeficient, sterile, sensitive to ionizing radiation, and prone to malignancies (Table I) [McKinnon, 2004]. A-T arises due to mutations in the ataxia telangiectasia mutated (*ATM*) gene.

Patients with ataxia telangiectasia-like disorder (A-TLD) have A-T-like phenotypes that stem from mutations in *MRE11*. The discovery that defects in Mre11, ATM, and Nbs1 cause similar cellular phenotypes (chromosome instability, DNA damage-dependent checkpoint defects, and radiosensitivity) was among the first indications that these proteins function in the same pathway, regulating the DNA damage response, and guarding against tumorigenesis.

Construction of *NBS1* and *RAD50* knockout mice revealed that the Mre11 complex is essential during early embryogenesis, a phenotype that precluded further analysis of Mre11 complex function [Luo et al., 1999; Zhu et al., 2001]. To surmount this problem, inducible systems were created to allow for mosaic and tissue-specific *NBS1* deletion. Disruption in B-lymphocytes led to the conclusion that Nbs1 functions in immunoglobulin class switching [Frappart et al., 2005; Kracker et al., 2005; Reina-San-Martin et al., 2005]. Interpretation of these results may be confounded by the fact that Mre11 complex function is essential in proliferating cells. Analysis of only live populations in these studies may circumvent this issue, but it is not known whether normal class switch functions can be maintained in cells that

TABLE I. Summary of the Phenotypes of Human Cancer Syndromes and Related Mouse Models

Human cancer syndrome	Human phenotype	Mouse model	Mouse phenotype
Nijmegen breakage syndrome (NBS)	Microcephaly, craniofacial abnormalities, skin hyperpigmentation, growth and mental retardation, impaired sexual maturation, gonadal failure (females), immunodeficiency, reduced IgG/IgA serum levels and pulmonary infection, increased cancer risk especially lymphoma	*Nbs1*$^{-/-}$	Embryonic lethal
		Nbs1$^{\Delta B/\Delta B}$	Only cellular phenotypes
		Nbs1$^{m/m}$	Growth retardation, gonadal failure (females), immunodeficiency, and lymphoma predisposition
		Nbs1 humanised	Impaired sexual maturation, gonadal failure (females) and reduced IgG/IgA serum levels
Ataxia telangiectasia (A-T)	Neurodegeneration, ataxia, telangiectasia, immunodeficiency, sterility, sensitivity to ionizing radiation, cancer predisposition	*Atm*$^{-/-}$	Growth retardation, immune defects, infertility, thymic lymphoma (around 4 months)
		Atm$^{\Delta SRI/\Delta SRI}$	Radiosensitivity, increased lifespan due to lower incidence of thymic lymphoma cf. *Atm*$^{-/-}$, tumor predisposition
Hereditary nonpolyposis colorectal cancer	Early onset colorectal cancer due to mutations in *MLH2*, *MSH2*, and *MSH6*	*Msh6*$^{-/-}$	Late onset colorectal cancer without microsatellite instability
Retinoblastoma (heterozygotes)	Loss of wt allele, retinoblastoma development at an early age	*Rb*$^{-/-}$	Embryonic lethal
		Rb$^{+/-}$	Loss of wt allele, pituitary and thyroid tumors
Li-Fraumeni Syndrome (p53 heterozygotes)	Predisposition to brain, breast, bone and soft tissue tumors, leukemia, adrenocortical, and gastric tumors	*p53*$^{-/-}$	Broad tumor spectrum at an early age, breast cancer only in BALB/c strain
		p53$^{+/-}$	

are undergoing events which will ultimately lead to their death. Study of this reaction in viable mutants or under non-proliferative conditions might address this problem.

The study of mosaic *NBS1* deletion using the nestin-cre mouse demonstrated a clear role for Nbs1 in proliferation and development of the cerebellum [Frappart et al., 2005; Betz, 1996 #7123]. The fact that deletion in other tissues of the developing mouse and the central nervous system did not result in gross defects is puzzling, but it is likely that only cells that maintain Nbs1 during proliferation survive. These cells may then differentiate and undergo *NBS1* deletion, giving the impression that Nbs1 is not essential in the development of these tissues.

Ultimately, the possibility that the cerebellar defect is proliferative cannot be ruled out, and the rare differentiated cerebellar cell types that are observed may arise from the few cells that maintain *NBS1* expression. Therefore, whether Nbs1 (or the Mre11 complex) has a function in homeostasis of the cerebellum can only be answered by deletion once development is complete. Whilst NBS patients do not exhibit ataxia or cerebellar defects like A-TLD patients, the answer to this question may shed light on whether ataxia in A-TLD (and A-T) patients stems from defects during development or degeneration of these tissues later on in childhood. Together, these examples illustrate the complexity of essential gene analysis and highlight the power of hypomorphic alleles to probe gene function.

Phenotypic Variability Between Models of the Same Disease

Frequently, a human disease is modeled by several groups using different approaches. Sometimes, as in the case with NBS models, this results in mice that recapitulate the disease and resemble each other to varying degrees. The majority of NBS patients identified to date harbor the *657del5* mutation (*Nbs1*^{657del5}), a 5 base pair deletion resulting in termination early in the *NBS1* open reading frame. This hypomorphic mutation results in production of an N-terminal fragment (p26) containing the Nbs1 FHA and BRCT protein interaction domains, and a C-terminal fragment (p70) containing the Mre11 interaction domain that is separately translated from a point downstream of the mutation [Maser et al., 2000].

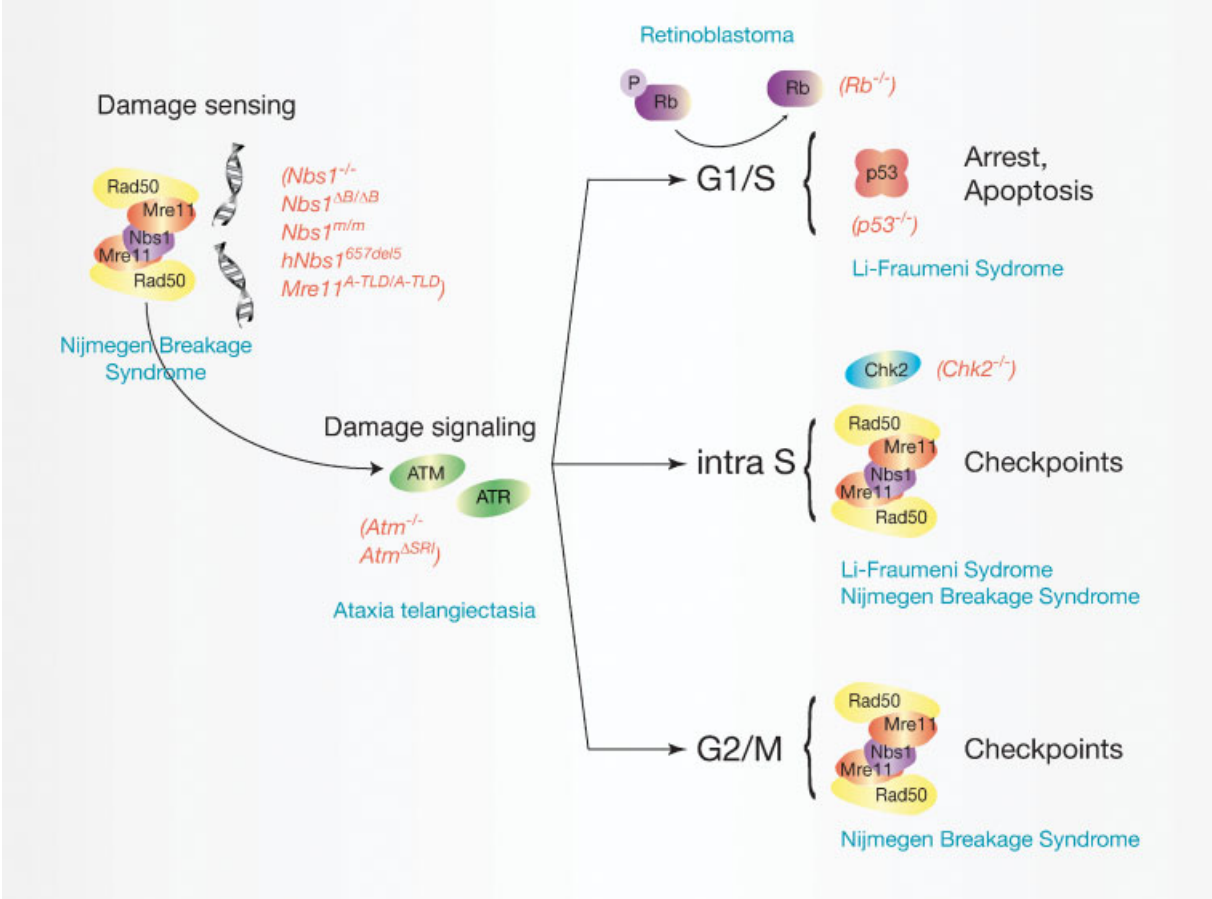

Fig. 1. Schematic representation of the DNA damage response pathway indicating steps in DNA damage recognition, human cancer syndromes (blue text), and mouse models discussed in this article (red text).

Unlike null mice, mice expressing alleles similar to $Nbs1^{657del5}$ are viable (Fig. 1). Replacement of exons 4 and 5 with Neomycin in the $Nbs1^{\Delta B}$ model results in production of a p70-like species initiated from sequence in the targeting construct upstream of exon 6 [Williams et al., 2002]. Deletion of exons 3 and 4 in the $Nbs1^{m}$ mouse also leads to expression of a p70-like species which is probably initiated from exon 4 or exon 7 [Kang et al., 2002]. Although these mutants closely model each other and NBS patients in some respects, (including defects in checkpoints and sensitivity and increased genomic instability in response to IR) some notable phenotypic differences are evident (see Table I). In $Nbs1^{\Delta B}$ mice, spontaneous chromosomal instability, immunodeficiency, and significant tumor predisposition are not observed. The $Nbs1^{m}$ mice do exhibit these phenotypes but do not exhibit microcephaly, and unlike NBS patients who are susceptible to B-cell lymphomas, primarily develop thymic lymphomas.

Rescue of $Nbs1^{-/-}$ mice using mutant human alleles has proved useful for probing the functions of various Nbs1 domains [Difilippantonio et al., 2005]. Expression of the human *657del5* allele in the null mice ($hNbs1^{657del5}$) resulted in a third NBS model. These mice have impaired sexual maturation in both genders, and complete ovarian dysgenesis in females. NBS patients exhibit similar phenotypes [Chrzanowska and Kanniger, 2002], and analysis of the $hNbs1^{657del5}$ mice revealed meiosis I progression is severely impaired. Like their human counterparts, these mice also exhibit reduced IgG serum levels and spontaneous genomic instability. Instability is especially evident in T-cells where translocations involving T-cell receptor (TCR) loci occur with high frequency. These types of translocations are used in humans for NBS diagnostic purposes, and $Nbs1^{\Delta B}$ and $Mre11^{A\text{-}TLD}$ mice exhibit a similar phenotype [Theunissen et al., 2003] suggesting that the Mre11 complex may be

directly involved in regulating *TCR* gene rearrangements.

NBS1 deficient mice rescued with human *Nbs1* containing an FHA inactivating point mutation exhibit many of the same phenotypes as the $hNbs1^{657del5}$ mice. This provides insight to NBS disease mechanism, suggesting that defects in patients and model systems may primarily stem from absence of the FHA domain. Like other NBS mouse models though, $hNbs1^{657del5}$ does not precisely recapitulate the human syndrome (Table I). For example, the $hNbs1^{657del5}$ mice are not prone to tumors. So far, two mice have developed tumors on a *p53* deficient background however, [Difilippantonio et al., 2005] perhaps suggesting that underlying genomic instability is kept in check by p53. Since p53 only partially suppresses tumorigenesis in $Nbs1^{m}$ mice [Kang et al., 2002, 2005], and evidence for p53 suppression of tumorigenesis in $Nbs1^{\Delta B}$ is lacking [Theunissen and Petrini, 2003], this illustrates yet another case in which phenotypic variability is observed between the different models.

One issue that may arise in systems using human alleles to rescue mouse deficiency stems from divergence between the human and mouse gene and protein. Differences between human and mouse Nbs1 (70% identity, 82% similarity) and gene regulatory sequences alone may lead to phenotypic outcomes. $hNbs1^{wt}$ mice generated for the sake of the $hNbs1^{657del5}$ studies did not exhibit any overt defects, indicating that the human allele efficiently compensates for the absent mouse gene, but absent long-term control studies, subtle effects cannot be excluded. Despite these potential drawbacks, the rescue of lethality in $Nbs1^{-/-}$ mice with mutant genes of human or mouse origin represents a powerful tool for probing gene function in the organism.

The observation that hypomorphic mutations in different Mre11 complex members do not phenocopy one another highlights an interesting paradox regarding the Mre11 complex and human disease phenotypes. Whilst similarities exist between A-TLD and NBS patients, including translocations in lymphocytes, and a number of cellular defects, the diseases are clinically distinct (for review see [Taylor et al., 2004]). For example, NBS patients never present with ataxia, which is thought to stem from cerebellar degeneration in A-TLD patients; and microcephaly and craniofacial abnormalities, defining characteristics of NBS, are not observed in A-TLD. The reason for these differences is not clear, but they could stem from a number of sources. Since NBS patients typically carry the *657del5* mutation, and consanguinity has been reported, it is likely that these patients are homozygous at many loci. This may lead to an increased effect of modifier alleles if they too are present in the homozygous state, and could explain the differences observed between NBS and A-TLD (see below for additional discussion on modifiers).

Perhaps the NBS and A-TLD phenotypic divergence is attributable to the fact that the *Mre11* and *Nbs1* hypomorphic mutants impair non-overlapping Mre11 complex functions. It is also possible that there are functions for the various complex members outside the context of the complex. Constitutive localization of Mre11 and Rad50 to telomeres, versus cell cycle-dependent localization of Nbs1 to telomeres during replication represents one example where Mre11 and Rad50 may have functions independent of Nbs1 [Zhu et al., 2000]. In support of this, synthetic lethality between $Mre11^{A\text{-}TLD1}$ and $Nbs1^{\Delta B}$ mice (our unpublished data) indicates that these mutations are not epistatic. Further study of these mouse models should help elucidate the reason for differences between the syndromes, and underscores the confounding problem that hypomorphic mutations in members of the same complex or pathway need not phenocopy each other.

Despite the incomplete phenotypic overlap of the mouse models between one another and in comparison to the human disease, use of these mutants to map genetic interactions between *NBS1* and genes such as *CHK2, ATM, H2AX*, and *p53*, have been instrumental to our understanding of the organization and hierarchy of the DNA damage response network proteins [Williams et al., 2002; Theunissen and Petrini, 2003; Theunissen et al., 2003; Difilippantonio et al., 2005; Kang et al., 2005]. Although the reasons for variability among mouse models of the same disease is not completely clear, the study of NBS mice illustrates how minor differences between alleles may account for some major phenotypic differences. The extent to which the p26 N-terminal Nbs1 fragment contributes to the human disease phenotype is currently unclear, and expression of this fragment in the mouse models has not been reported, leaving the possibility open that the

presence or absence of this fragment accounts for some of the phenotypic differences.

The extent to which other factors, such as modifiers and genetic background, affect these outcomes is relatively unknown and will be discussed in further detail below. The fact that NBS model systems typically display less severe phenotypes than those of NBS patients raises the question of whether fundamental differences exist between the mouse and human DSB response. Alternatively, conditions of environmental stress may be sufficiently low in the laboratory setting as to result in the absence of some phenotypic outcomes that would be readily apparent under more normal conditions.

Allele-Specific Phenotypic Variability

Another well-studied model of a human cancer syndrome is the *ATM* knockout mouse. The product of *ATM* is a protein kinase that is activated upon DNA damage and activates signal transduction pathways that initiate cell-cycle arrest, repair, and apoptotic programs (for reviews see [Bakkenist and Kastan, 2004; Kastan and Bartek, 2004]). As described above, homozygous *ATM* mutations result in ataxia-telangiectasia (A-T) (Fig. 1). Interestingly, individuals heterozygous for *ATM* do not display A-T phenotypes, although they may be predisposed to cancer, in particular, to solid tumors such as breast cancer [Swift et al., 1986, 1987; Easton et al., 1993].

Unlike NBS models, $Atm^{-/-}$ mice display the same phenotypes as A-T patients, recapitulating many of the clinical features of A-T and dying of thymic lymphomas at an early age (frequently before 4 months) (Table I) [Barlow et al., 1996; Xu et al., 1996]. However, one of the most puzzling differences between the human syndrome and mouse models is the lack of tumor formation in *ATM* heterozygous mice, despite their increased radiation sensitivity [Barlow et al., 1999; Weil et al., 2001]. An explanation for this discrepancy was suggested when various *ATM* missense mutations identified in A-T or breast cancer patients were shown to behave in a dominant-negative manner upon re-introduction into wild-type control cells [Scott et al., 2002]. Elegant studies using a knock-in strategy corroborated this result in mice [Spring et al., 2001, 2002]. These studies generated mice carrying a 3 amino acid deletion in *Atm* ($Atm^{\Delta SRI}$), mimicking one of the most common deletion mutations in humans ($ATM^{7636DEL9}$) that results in an increased cancer frequency in heterozygous patients (Table I). Heterozygous knock-in mice carrying this mutation also show an increased cancer frequency and, $ATM^{7636DEL9}$ cDNA was shown to exhibit a dominant negative effect when expressed in control cells, inhibiting radiation-induced ATM kinase activity [Spring et al., 2002]. These data suggest that $ATM^{7636del9}$ is indeed a dominant negative allele.

This supports a model proposed by Gatti et al., who predicted that two different classes of *ATM* mutations give rise to distinctive tumor predispositions in the human heterozygous population [Gatti et al., 1999]. The first class comprises null or truncation mutations that essentially result in loss of detectable protein from this allele, mutations that *ATM* null mice directly model. The second class was thought to represent mutations in which the protein is expressed but behaves as a dominant negative, interfering with the function of the remaining wild-type allele (e.g., point mutations or deletions/insertions). Thus in the first class, heterozygotes have reduced *ATM* dosage but one functional wild-type allele, whilst the second class of heterozygotes are essentially *ATM* null and are therefore cancer prone.

This work highlights the potential of the knock-in strategy for gaining insight into allele-specific disease etiology. Interestingly, $Atm^{\Delta SRI}$ mice have a significantly longer lifespan than $Atm^{-/-}$ mice, probably due to a lower incidence of thymic lymphoma in the dominant negative mouse model [Spring et al., 2001]. Thus the proposed dominant negative mutation does not give rise to a truly null phenotype and may imply some residual function of the wild-type Atm protein. In fact, this reflects the human disease, where *ATM* homozygous mutant individuals show a more severe phenotype than *ATM* heterozygotes [Angele and Hall, 2000; Khanna, 2000]. Further study of this allele and the generation of accurate models of other dominant negative *Atm* alleles should provide insight into the mechanisms involved in tumorigenesis.

Penetrance

An issue emerging from studies on the effects of *Atm* heterozygosity in humans has been that of penetrance, a factor that is difficult to assess in mouse models. As discussed previously, *ATM* heterozygous individuals have been reported to

show an increased predisposition to breast cancer development [Swift et al., 1986, 1987; Easton et al., 1993]. Based on the estimated frequency of heterozygous carriers in the population, *ATM* was therefore predicted to be one of the major genetic players in breast cancer development [Hopper and Carlin, 1992]. However, analysis of patients with breast cancer has revealed a far lower rate of *ATM* mutations than expected. One early study that screened for protein truncations in patients with early onset breast cancer relative to control subjects did not find any increase in A-T carriers [FitzGerald et al., 1997]. Similar results were obtained when radiosensitive breast cancer patients were compared with control individuals [Appleby et al., 1997; Ramsay et al., 1998; Shayeghi et al., 1998]. These results have been put into perspective somewhat by the observation that the type of mutation plays a key role in cancer predisposition of heterozygotes. Studies that have screened for amino acid substitutions or in-frame deletions/insertions, rather than protein truncations, have in fact identified increased frequencies of A-T mutations relative to controls ([Izatt et al., 1999] and see [Khanna, 2000]). Thus the penetrance of A-T heterozygous phenotypes is dependent on the type of mutation present. However, it is clear that the observed penetrance of an allele can also be biased by the population subset chosen for the epidemiological study.

Mouse models can be instrumental in uncovering low penetrance genes, as exemplified by *MSH6* mutations. Hereditary nonpolyposis colorectal carcinoma (HNPCC) arises primarily due to mutations in mismatch repair genes. HNPCC is a highly penetrant syndrome characterized by early onset colorectal cancer (<45 years), with an estimated lifetime risk of colorectal cancer development of 85–90% (reviewed in [Lynch and de la Chapelle, 1999, 2003]). The most commonly identified mutations that give rise to HNPCC are found in the mismatch repair genes *MLH1* and *MSH2*, and contribute to approximately 90% of cases of HNPCC [Lynch and de la Chapelle, 2003]. Msh2 functions together with Msh6 to recognize base:base mispairs and insertion/deletion mispairs, thus it was surprising that germline *MSH6* mutations were not also identified in cases of HNPCC [Liu et al., 1996].

The generation of *MSH6* knockout mice showed that loss of this gene does, in fact, cause cancer susceptibility, but without microsatellite instability, commonly used as a phenotypic determinant for HNPCC (Table I) [Edelmann et al., 1997]. As a result, many individuals with germline *MSH6* mutations may have been excluded from HNPCC studies due to lack of microsatellite instability. Following the *MSH6* knockout report, germline *MSH6* mutations were in fact identified as being causative for HNPCC, but were found to predispose to late-onset colorectal carcinomas that do not fulfill the classic criteria for HNPCC [Kolodner et al., 1999]. The late onset of these carcinomas (median age 61) is only slightly lower than the ages for all cases in the United States (median age 68), and is 10 to 20 years older than the usual median age at diagnosis of HNPCC. Indeed, this late onset certainly contributed to the difficulty in identifying *MSH6* mutations as causative for HNPCC prior to the generation of a null mouse model. To date, *MSH6* mutations have been identified in approximately 10% of HNPCC cases [Lynch and de la Chapelle, 2003]. Thus these mutations demonstrate how differences in penetrance affect the phenotypic outcome and, in this case, how the use of mouse models allowed the elucidation of a lower penetrance gene that contributes to the familial cancer syndrome.

Strains and Genetic Modifiers

Another factor that can affect the observed penetrance of mutations, and that is vastly different between human and mouse models, is that of genetic background. Whilst the human population is genetically heterogeneous, most laboratory mice have been bred to obtain "pure" (i.e., homogenous) genetic backgrounds. Even mice considered to be of "mixed" genetic background are relatively homogeneous, as they generally arise from a limited degree of outbreeding. Given the contrasting genetic constitutions of laboratory mice and the human population, it is useful to consider the limitations posed by recombinant inbred strains in modeling disease endemic to the human population.

The tumor suppressor retinoblastoma, *RB*, is one of the most frequently mutated genes in all human tumors [Sherr and McCormick, 2002]. The pRb protein is a critical regulator of cell cycle progression and its loss results in cell cycle deregulation, unscheduled proliferation and, ultimately, tumorigenesis. Thus deregulation

of the pRb tumor suppressor pathway appears to be an essential step in tumor progression [Classon and Harlow, 2002]. Patients with heterozygous germline *Rb* mutations show loss of heterozygosity (LOH) of the remaining wild-type allele, and develop retinoblastoma in early childhood with extremely high penetrance (Fig. 1 and Table I).

Loss of *Rb* in mice is lethal at days 13–15 after gestation, due to placental defects that restrict the exchange of oxygen and nutrients between mother and fetus [Wu et al., 2003]. Heterozygous mice are viable but rapidly develop tumors with nearly 100% incidence, thus mimicking human tumorigenesis (Table I). $Rb^{+/-}$ mice do not develop retinoblastoma but show an increased predisposition to pituitary and thyroid tumors [Harrison et al., 1995]. A recent study on the penetrance of *Rb* heterozygosity in serially backcrossed 129Sv versus C57BL/6 background mice showed that this may be a strain-dependent effect, as C57BL/6 $Rb^{+/-}$ mice show significantly increased survival [Leung et al., 2004]. Furthermore, this study found that 129Sv mice possess an inherently abnormal intermediate lobe of the pituitary gland (ILP) that enhances the initiation and progression of ILP tumorigenesis. This irregularity may account for the high incidence of tumors in this strain, which rarely occur in wild-type (mixed background) mice, and may explain the incidence of these tumors in other knockout mouse models [Nakayama et al., 1996; Franklin et al., 1998].

Similar observations have been made for the *p53* knockout mouse. *p53* is a tumor suppressor gene that is frequently mutated in human tumors, with germline *p53* mutations giving rise to the autosomal dominant cancer predisposition syndrome, Li-Fraumeni Syndrome (LFS) (Fig. 1) [Varley, 2003]. Loss of *p53* in mice is not lethal but they develop a broad spectrum of tumors at an early age (Table I) [Donehower et al., 1992]. The predominant tumor type seen in mice of a mixed background is lymphoma, which also predominates in a pure 129Sv background. However, pure 129Sv $p53^{-/-}$ mice also develop testicular tumors (primarily teratocarcinomas) with high incidence (~50% in 129Sv compared to 10% in a mixed background), indicating that some tumor types may be strain-dependent [Harvey et al., 1993].

Perhaps more significantly, *p53* mutations are found in a large proportion of sporadic and familial breast cancers, yet mammary tumors are not seen in *p53* null mice [Schuyer and Berns, 1999]. This has now been shown to be due to an apparent resistance to mammary tumor formation in the 129 and C57BL/6 backgrounds. On a BALB/c background, the predominant tumor type for $p53^{-/-}$ mice remained malignant lymphoma. However, 55% of *p53* heterozygous animals developed mammary carcinoma, a tumor type not seen in the 129 and C57BL/6 backgrounds. This suggests a predisposition for this tumor type on the BALB/c genetic background and potentially provides a model system in which to study p53 mutations and breast cancer [Kuperwasser et al., 2000].

Genetic modifiers are also known to affect tumor predisposition in both mice and humans. The use of mouse models of pure genetic background, whilst not representative of the diverse human population, allows the potential identification of genetic modifiers through linkage analysis. This information can then be applied to human disease to attempt to explain differences in penetrance between individuals and may ultimately lead to idiosyncratic "personalized" therapies.

One excellent example of a genetic modifier is Modifier of Min1, Mom1. The Min mouse (multiple intestinal neoplasia) models human familial adenomatous polyposis (FAP), a cancer predisposition syndrome characterized by the development of adenomatous polyps in the colon [Rowley, 2005]. Significantly, FAP displays variable phenotypic expression that is linked to both the genotype (mutations within the gene adenomatous polyposis coli (*APC*)), and the influence of modifier genes [Crabtree et al., 2002]. The existence of genetic modifiers was first shown through studies of the Min mouse, in which the gene for secretory type II phospholipase A2 (*PLA2S*) was identified as Mom1 [MacPhee et al., 1995]. *PLA2S* has since been found to confer an active non-autonomous resistance to tumorigenicity, thus acting as a genetic modifier of *APC* as predicted [Dove et al., 1998]. To date however, no variants have been found in the human homolog, *PLA2G2A*, that are predicted to result in functional variants [Tomlinson et al., 1996]. Thus *PLA2G2A* does not appear to be a genetic modifier for human disease and other genetic modifiers remain to be identified.

Recent studies in the mouse have now identified the Mom2 locus although the gene

involved currently remains elusive [Silverman et al., 2002; Silverman et al., 2003]. Mom2 is of particular interest for human cases of FAP as its locus in the mouse genome is syntenic with human chromosome 18q, a region that frequently undergoes loss of heterozygosity (LOH) in human cancers. Thus the identification of the gene responsible for Mom2 may aid in the assessment of FAP, as individuals may be tested for mutations in APC in conjunction with Mom2. Based on these results, an assessment of the relative risk can be made and, if necessary, individuals may then undergo early screening for the presence of polyps. Understanding the effects of genetic modifiers is, therefore, directly applicable as it may identify mutations that put individuals at higher risk of tumor formation.

Mice can therefore be instrumental in understanding and identifying genetic modifiers. However, when studying mouse models it is also worth considering that genome-wide homozygosity of laboratory strains may bias for the detection of mild-genetic modifiers. In contrast, in a genetically hetergeneous context such as in humans, low penetrance modifiers are less likely to be detected (e.g., due to heterozygosity with non-modifying alleles), and only highly penetrant modifying alleles are likely to influence phentoypic outcomes.

Compensation

The issue of compensation is not unique to mouse models, but stark examples in the mouse highlight a fundamental problem posed by using genetic deficiency in pathway analysis. Complete loss of a gene is likely to result in the loss of more than one protein function and may affect several cellular pathways. Loss in embryogenesis may allow related proteins to functionally compensate for the one that has been lost resulting in a modified, usually less severe phenotype. In contrast, loss of a protein may not give a phenotype if a protein with an overlapping function exists, or if a related protein can adopt the function of the deleted gene product (compensation). Compensation may only be possible if a protein is required to adopt a novel function in embryogenesis, that is, in a situation in which selection is possible. The same protein may not functionally compensate if the related protein is lost in an acute (conditional) setting. Thus the generation of conditional null models may distinguish between compensation and functional overlap.

Knock-in models (deletions, truncations, point mutations) allow a specific function of the protein to be targeted whilst retaining other wild-type functions. In this way, it is possible to preserve some cellular pathways and to look more specifically at the effect of loss of a particular function. Thus, the data obtained may be more informative since the changes within the cell are likely to be more specific. Ultimately, the use of both null and conditional models together is likely to be the most informative approach to understanding both global and specific protein functions.

One example in which compensation has been definitively shown to affect the phenotype of knockout mice is the *Rb* knockout. As described previously, *Rb* heterozygous mice develop tumors with extremely high incidence but the tumor type does not reflect that seen in cases of human retinoblastoma. This has now been shown to be due to compensation by two related proteins from this protein family (known as pocket proteins), p107 and p130. Although loss of *Rb* is lethal following the intercrossing of two heterozygous ($Rb^{+/-}$) animals, chimeric $Rb^{-/-}$ mice can be obtained by injecting $Rb^{-/-}$ ES cells into blastocysts, where the presence of the wildtype cells apparently protects against lethality [Maandag et al., 1994]. Although $Rb^{-/-}$ chimeras display developmental defects in the eye, as with *Rb* heterozygotes, these animals do not develop retinoblastoma. Generation of $Rb^{-/-}$ chimeras with concomitant loss of *p107* leads to a high incidence of retinoblastoma [Robanus-Maandag et al., 1998]. This elegant work provided the first evidence that, in mice, p107 acts as a suppressor of retinoblastoma by compensating for some of the functions of pRb. A more recent study in mice confirmed this showing that conditional mutation of *Rb* in the developing nervous system results in retinal dysplasia and retinoblastoma only on a *p107*- or *p130*-deficient background, respectively [MacPherson et al., 2004].

Proof of compensation by p107 has also been provided in tissue culture using conditional *Rb* mouse embryonic fibroblasts (MEFs). This study showed that both germline $Rb^{-/-}$ and conditional $Rb^{-/-}$ MEFs proliferate and arrest normally due to compensatory upregulation of *p107*. However, acute loss of *Rb* in quiescent cells results in cell cycle re-entry without upregulation of *p107* suggesting that compensation cannot occur in conditions where Rb is

playing an active role in maintaining the cell cycle state [Sage, J. Nature, 2003]. Consistent with this, acute loss of *Rb* in senescent cells also results in cell cycle re-entry, showing that compensation by p107 is context dependent. Thus it appears that retinoblastoma is not seen in the *Rb* mouse due to compensation by related proteins, and that either species- or context-specific differences may account for the varying phenotypes between human and mouse. This predicts that the human homologs of these proteins are unable to compensate for the specific pRB function that suppresses retinoblastoma.

Tissue Specificity

Mouse models may also differ from human cancer syndromes in their tissue specificity. As discussed in the case above regarding *RB* heterozygosity, these mice do not develop the retinoblastoma that is seen in affected human carriers, but die of pituitary or thyroid tumors with approaching 100% incidence. This tissue specificity appears to be affected both by compensation by related family proteins, and by a strain-specific inherent predisposition to another tumor type- intermediate lobe pituitary tumors.

Similar observations have been made for NBS mouse models, where humans develop lymphomas, primarily of the B-cell type, whilst $Nbs1^{\Delta B/\Delta B}$ mice are not predisposed to tumor development and $Nbs1^{m/m}$ present mainly with thymic lymphomas. There are many other examples of mouse models in which either the tumor type or the location of origin does not mimic that predominantly seen in humans. Li-Fraumeni Syndrome, usually associated with germline *p53* mutations, is characterized by a specific tumor spectrum consisting of brain, breast, bone and soft tissue tumors, leukemias, and adrenocortical and gastric tumors. As discussed in the Strains and Genetic Modifiers section, the *p53* knockout mouse is prone to tumorigenesis but the tumor spectrum differs from that seen in humans, consisting predominately of malignant lymphoma, and with the absence of mammary tumors [Donehower et al., 1992]. This may be, in part, due to strain-specific differences (as discussed above); however because most of the human *p53* mutations are missense mutations, it may also reflect the difference between null and hypomorphic mutational outcomes. The recent generation of *p53* knock-in mice harboring one of the mutations most frequently seen in human tumors ($p53^{R175H}$) has highlighted this fact, as these mice develop fewer lymphomas and more carcinomas, with a higher degree of metastasis, more closely modeling LFS [Liu et al., 2000].

The protein kinase CHK2 is mutated in the germline in some patients with LFS [Bell et al., 1999], and sporadic CHK2 mutations have also been identified in patients with breast cancer [Meijers-Heijboer et al., 2002; Vahteristo et al., 2002]. Yet *Chk2* knockout mice show no predisposition to cancer development (Fig. 1) [Hirao et al., 2002; Takai et al., 2002]. This phenotypic difference may be due to the different genotypes—total loss in the mouse compared to a truncation in humans—again, suggesting the merit of a knock-in model.

Thus, although mouse proteins may carry out the same function as their human homologs, the phenotypic outcome, in particular tissue and tumor-specific phenotypes, can vary greatly between the two species. As such, awaiting tumor development in order to analyze gene-specific phenotypes may not be the most efficient approach. An alternative system is to use meiosis to study the effects of disruption of members of the DNA damage response pathways. As described below, meiosis provides a synchronous, accessible system in which to follow the repair of DNA double strand breaks.

MEIOSIS: AN ENDOGENOUS DSB RESPONSE SYSTEM

Study of mammalian meiosis has become accessible due to the accumulation of an extensive body of knowledge regarding the events that take place during mouse gametogenesis and their genetic dependencies, including the definition of many architectural transitions and molecular events. Furthermore, its relatively slow, and synchronous progression in juvenile males make it an excellent system for asking questions regarding the timing of a particular gene function in the meiotic process.

Repair of meiotic DSBs represents a controlled response to programmed damage, and therefore provides the advantage of studying repair in a physiological setting versus damage from an exogenous source. Meiotic findings might foreshadow mitotic interactions that take much longer to assess, for example, in cases

where tumor susceptibility is monitored, or reagents such as primary or immortalized cell lines need to be derived before experimentation.

Meiosis is the specialized cell division that occurs in sexually reproducing organisms to produce gametes of the appropriate ploidy. The process by which meiotic cells undergo one round of replication followed by two sequential rounds of division is conserved from yeast to humans (for a detailed review of this subject see [Roeder, 1997; Zickler and Kleckner, 1999; Marston and Amon, 2004; Richardson et al., 2004]). The first round of meiotic cell division (meiosis I) is punctuated by three unique events: catalysis of many DSBs by the topoisomerase-like enzyme Spo11, the alignment and synapsis of homologous chromosomes via the formation of a proteinacious structural element called the synaptonemal complex (SC), and the formation of crossovers between homologous chromosomes (for reviews on these topics see references above, and [Keeney, 2001; Page and Hawley, 2004]). Crossovers constitute the physical connection between homologs once the SC is removed, and allow for their biorientation along the metaphase plate, and their segregation at the end of meiosis I.

Underscoring the basic link to chromosome biology of both tumor suppression and meiosis is the fact that many patients with increased cancer susceptibility syndromes exhibit infertility [German, 1969; Boder, 1975; Group, 2000; Chrzanowska and Kanniger, 2002]. In recent years, it has become evident that the underlying cause of pleiotropic outcomes such as these, stem from defects in signaling and repair network proteins which have a role in both the mitotic and meiotic program. Mutations in mitotic damage response proteins such as Atm, Nbs1, H2AX, Mlh1, and Brca1 lead to meiotic defects, suggesting that many damage response functions may be conserved in meiosis [Barlow et al., 1998; Celeste et al., 2002; Difilippantonio et al., 2005; Edelmann et al., 1996; Xu et al., 2003].

One mutant that has been extensively studied in meiosis is *Atm*. $Atm^{-/-}$ mice, like their human counterparts, are infertile [Barlow et al., 1996]. This is due to a defect in prophase of meiosis I, resulting in p53-dependent and independent elimination of meiosis I cells [Barlow et al., 1997]. Detailed cytological analysis of spermatocytes from these mice revealed aberrant Rad51 and Dmc1 localization in leptotene and early zygotene cells, failure to disperse meiotic telomere clusters (which normally occur early in prophase and are thought to be critical for pre-alignment of homologous chromosomes), and defects in homologous synapsis [Barlow et al., 1997, 1998; Pandita et al., 1999]. These results indicate that ATM-mediated damage signaling is critical for coordination of DSB repair events with meiotic progression, which likely results in synaptic failure.

Interestingly, reduction in dosage of Spo11 partially rescues the meiotic progression defects of $Atm^{-/-}$ mice [Bellani et al., 2005]. This suggests that other proteins might be able to compensate for ATM when damage load is reduced, or that trigger of meiotic arrest depends on reaching some threshold of unrepaired lesions that is not achieved when Spo11 dosage is reduced.

In meiosis, a seemingly paradoxical function for ATM has emerged: ATM activity is required for arrest of mitotic cells in response to damage, but its absence in damaged meiotic cells compromises progression. An appealing reconciliation of this apparent paradox is that the checkpoint pathways in both mitotic and meiotic cells directly influence DNA repair. Hence, the effect of checkpoint pathways on meiosis may not require members of the pathway that regulate cell-cycle progression. Analysis of non-null ATM mutations in meiosis, or expression of mutant alleles in the ATM deficient background (such as kinase dead ATM) could be examined for rescue of $Atm^{-/-}$ meiotic phenotypes. If rescue is evident, this might be attributable to functions of ATM aside from events regulated by its kinase activity.

A requirement for checkpoint proteins in meiotic progression may also stem from the fact that meiosis I is not a cell cycle per se, but rather the specialized prophase and metaphase of a cell that undergoes a unique reductional division. It is therefore likely that checkpoint signaling pathways in meiosis converge on largely different meiosis-specific endpoints, which are currently not well understood. Elucidation of these signaling pathways through the generation of hypomorphic, conditional or null alleles, including those that model human damage response gene mutations, will provide insight to mammalian meiosis and the specific defects that give rise to reproductive phenotypes.

CONCLUSIONS AND FUTURE DIRECTIONS

Mouse models of human cancer syndromes have resulted in enormous advances in our understanding of many DNA damage and cell-cycle pathways. To date, these findings have contributed greatly to current screening approaches and are being used to develop more specific anti-cancer drugs. With the development of ever more advanced mouse models, we are sure to gain further insights into tumor biology. Whilst the study of mutations in a different species is not perfect, awareness of factors that may affect the observed phenotype, and consideration of possible outcomes, is generally sufficient to ensure valid interpretation of the in vivo data. However, as highlighted above, meiosis may be an alternative system in which to initially study the effects of genetic change in a rapid and accessible system that, in particular, precludes the wait for tumor development.

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