Closing the gaps among a web of DNA repair disorders

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Summary
As recently as six years ago, three human diseases with similar phenotypes were mistakenly believed to be caused by a single genetic defect. The three diseases, Ataxia-telangiectasia, Nijmegen breakage syndrome, and an AT-like disorder are now known, however, to have defects in three separate genes: ATM, NBS1, and MRE11. Furthermore, new recent studies have shown now that all three gene products interact; the ATM kinase phosphor-ylates NBS1, which, in turn, associates with MRE11 to regulate DNA repair. Remarkably or expectedly, depending on one’s point of view, the similarity in disease phenotypes is evidently due to defects in a common DNA repair pathway. BioEssays 22:966–969, 2000. © 2000 John Wiley & Sons, Inc.

Introduction
Discoveries in biology are exciting either because they are surprising, or because they simplify the once bewildering. Several recent findings about a number of human disease genes involved with DNA repair seem exciting for both reasons. First it was discovered that ATM, p53 and CHK2 act in the same pathway, and now new findings show that ATM, NBS1 and MRE11 form part of another interesting pathway. It is remarkable, even to the point of provoking the envy of yeast geneticists, that human genetics neatly provides such mutants in genes acting in common pathways. A simplifying explanation of the function of these disease genes seems close at hand, given recent discoveries showing specific interactions between ATM and NBS1, published in recent issues of Nature and Nature Genetics. We discuss these findings that provide a molecular explanation for the similarities among several genetic diseases, as well as the observation of interactions with yet another disease gene, BRCA1. The conclusions are summarized in Fig.1, which provides a guide for the discussion that follows.

AT and NBS history
Ataxia-telangiectasia (AT) is a human autosomal recessive disorder manifesting many symptoms. These include immune deficiency, cerebellar degeneration, premature aging, radiation sensitivity, genomic instability, and an increased predisposition to cancer.(5) The disease gets its name from the devastating neuromotor dysfunction (ataxia) and dilated blood vessels of the eye (telangiectasia). Another characteristic feature of AT is the development of tumors; it is estimated that about 10–15% of AT patients develop a malignancy at an early age.(6,7)

Nijmegen breakage syndrome (NBS) presents a very similar clinical picture. NBS patients also display symptoms of radiosensitivity, immune dysfunction, genomic instability and cancer predisposition. Interestingly, however, they do not develop neuronal degeneration and therefore, are not plagued with the motor defects observed in AT.(6) Given their similar clinical manifestations, NBS was long considered an “AT variant.” This view lasted until 1995, when it was demonstrated that mutation of the ATM (Ataxia-Telangiectasia Mutated) gene is responsible for all the phenotypes of AT,(8) while the ATM gene was found to be intact in NBS individuals. Nonetheless, the similar phenotypes of affected AT and NBS individuals strongly suggested that the diseases were functionally related.

Molecular relationships among ATM, NBS1 and MRE11
Several landmark papers in 1998 and 1999 set the stage for the current findings that establish the basis for similarities between AT and NBS. First, Varon et al.(9) cloned the gene responsible for NBS (NBS1). They found that the NBS1 protein contained two domains, a BRCT and a FHA domain, which in other proteins have roles in DNA damage repair and in cell cycle checkpoints. Next, it was inferred that NBS1 was required for the RAD50/MRE11 DNA repair complex involved in repair of double strand breaks (DSBs); the formation of RAD50 and MRE11 protein clumps, or foci, at presumptive sites of DNA breaks in cells was defective in NBS1 mutant cells.(10) Finally, it was discovered that patients with the rare “AT-like disorder” (AT-LD) have mutations in the MRE11 gene.(11) AT-LD and NBS patients have very similar phenotypes and the corresponding proteins are now known to act together as a molecular complex in DNA repair.(10)
pressing NBS1 mutant proteins that cannot be phosphorylated the phosphorylation has specific consequences. Cells ex-

Two additional features of the phosphorylation of NBS1 deserve special emphasis. First, ATM is only required for very rapid phosphorylation of NBS1 following IR (and formation of DSBS). In AT cell-lines (cells lacking functional ATM protein), phosphorylation in the first few hours is lost, but is completely evident at six hours and beyond. This is very similar to reports of p53 and CHK2 phosphorylation, which confirm that ATM is also necessary for immediate phosphorylation following IR, but not beyond six hours. Second, phosphoryla-
tion of NBS1, as well as of p53 and CHK2, after exposure to other forms of DNA damage (UV or depletion of dNTPs due to hydroxyurea (HU)), is entirely ATM-independent, and must therefore occur by a separate protein kinase.

The most likely protein kinase to modify NBS1, CHK2 and p53 following damage by UV and HU (as well as a delayed
response to IR) is the ATM homologue, ATR (ATM-Rad3-related). (19,20) Considerably less is known about ATR, partly due to the absence of known human ATR mutant syndromes and the fact that ATR is essential for cell viability. (21) Why two such similar protein kinases have evolved and are retained in the genome remains speculative, though one can imagine that each kinase may be activated by different types of DNA lesions; irradiation generates DSBs, while HU and UV would seemingly generate single strand gaps, for example. How different types of damage might activate different kinases has not been illuminated by study of the corresponding yeast protein kinases, since both Rad3 in S. pombe and Mec1 in S. cerevisiae respond to all types of damage. (13,22) In fact, Rad3 and Mec1 share significantly more functional homology (and slightly more protein sequence homology) with ATR than with ATM. (19) so an interesting question is why ATM has only evolved in multicellular organisms? The answer is currently unknown, but it is clear that multicellular organisms are less proficient at repairing DSBs than either species of yeast, probably due to relatively weak homologous recombination capacity. Perhaps ATM represents a gene duplication of ATR as a form of “specialized DSB checkpoint and repair gene” to provide immediate response to particularly dangerous lesions (DSBs). Since, DSBs might occur spontaneously during replication, this would explain why AT cells suffer genomic instability and AT patients are at a higher risk for malignancies.

Ever-more insights from human diseases

The study of human disease genes continues to spark interest in DNA repair and provide direction for future research. The number of distinct genes acting in common pathways seems remarkable. P53 and CHK2 are both associated with similar genetic diseases with dramatic predilections to cancers (Li Fraumeni syndrome). (23,24) The relationships among ATM, NBS1 and MRE11 have been discussed here. Another human disease gene with activities that may be associated with ATM, NBS1 and MRE11 is the breast cancer susceptibility gene, BRCA1. BRCA1 is also a substrate for ATM and the picture is again comparable to that of p53 and NBS1; where ATM phosphorylates BRCA1 following IR (DSBs) but the phosphorylation is independent of ATM after UV damage or HU treatment. (25) BRCA1 also interacts with DNA repair proteins (particularly RAD51) and cells deficient in BRCA1 have defects in DNA repair activity. (26–28) Whether BRCA1 protein also functionally interacts with NBS1, MRE11, and RAD50 is unclear. BRCA1 may affect DNA repair by recruitment of the R/M/N complex to DNA breaks, although this is currently a hot area of debate (see online discussion in Ref. 29). Another group has suggested that BRCA1 is actually the molecular “glue” that holds virtually all known DNA repair proteins together, and that this huge complex acts as a multipurpose DNA repair machine, which they call BASC (BRCA1-associated genome surveillance complex). (30) Whether all these repair proteins perform their activities as a part of BASC in vivo remains to be seen.

One last point regarding ATM function arises from the recent finding that MRE11 mutations are responsible for AT-LD. (11) The specific neurodegenerative symptoms associated with AT are difficult to explain considering ATM’s only known functions are general ones, in checkpoints and DNA repair. One theory is that ATM acts as a sensor of oxidative damage, which can be especially prevalent in neurons, and in ATM’s absence the result of oxidative damage could be apoptosis. (31) Many others have considered the neuronal degeneration to be caused by loss of a currently unknown ATM function that is separate from its roles in genomic integrity. However, similar neurodegenerative phenotypes are also observed in patients with mutant MRE11. Therefore, MRE11 may also play a role in preventing oxidative damage or a simple explanation might be that neuronal cells are particularly susceptible to dysfunctional DNA repair pathways.

Epilogue

As yeast geneticists reviewing progress on human disease genes, we surprise ourselves even to imagine that we are asking the following questions. How successful has the study of human diseases been in identifying major DNA repair pathways? Why have genes in the pathways shown in Fig. 1 been repeatedly identified? Could these pathways, indeed, represent a large part of all possible repair pathways, along with NER, BER and MMR? Alternatively, are there major repair pathways in higher organisms still uncharacterized, perhaps because they perform more essential roles (such as may be the case for the ATR gene)? Are we looking only where the light shines, or is a comprehensive understanding of DNA repair pathways (and of cell cycle checkpoints) actually close at hand? Obviously, there is still a considerable amount of work to do in figuring out how all the components of the known pathways perform their functions. Yet, we may already know the essentials of most repair pathways. If so, we can soon look forward to fulfilling the major purpose of their elucidation—how can they be manipulated for medical benefit?

References


