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 phosphorylates NBS1,[1–4] 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.

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*.[1–4] 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.

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Abbreviations: ATM, Ataxia-telangiectasia-mutated; NBS, Nijmegen breakage syndrome; DBSs, double-strand breaks; IR, ionizing radiation; AT-LD, AT-like disorder; RDS, radioresistant DNA synthesis; ATR, ATM-Rad3-related; HU, hydroxyurea; NER, nucleotide excision repair; BER, base excision repair.

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]

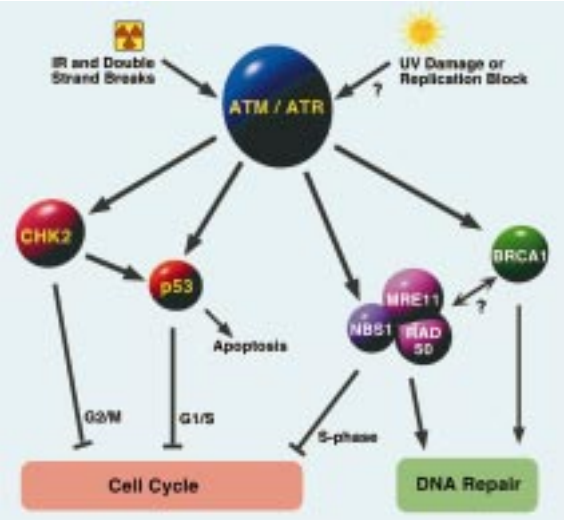

Figure 1. A model for *ATM* regulated genes. When a DNA double-strand break (DSB) occurs, such as by ionizing radiation (IR), ATM phosphorylates a combination of proteins responsible for cell cycle arrest and DNA repair. Phosphorylation of p53,[18] NBS1[1–4] and CHK2[17] induce individual cell cycle checkpoints. Phosphorylation of NBS1 also enhances the DNA repair complex of RAD50/MRE11, and this could be modified by BRCA1. After UV damage or a block to DNA replication, the ATR kinase might replace ATM for phosphorylation of overlapping substrates. For simplicity, the number of arrows in the figure do not represent all possible interactions. For example, BRCA1 is also a substrate of CHK2.[32] Mutations in most of the genes shown have been implicated in human disease, including: *ATM*/Ataxia-telangiectasia, *NBS1*/Nijmegen breakage syndrome, *P53* and *CHK2*/Li Fraumeni syndrome, *MRE11*/Ataxia telangiectasia-like disorder and *BRCA1*/familial breast and ovarian cancer.

In hindsight, the strong similarities in clinical and cellular phenotypes of AT, NBS and AT-LD presaged what has come next, the discovery that ATM and the RAD50/MRE11/NBS1 (R/M/N) complex function in the same pathway. It had previously been found that the ATM protein is a member of the PI-3 like protein kinase (PILK) family, which responds to DNA damage by signaling cell cycle arrest and modulating DNA repair.[6,12,13] Four research laboratories now report that ATM modifies the activity of the R/M/N complex by phosphorylating NBS1.[1–4] Following exposure to ionizing radiation (IR), there is rapid phosphorylation in vivo of at least two serine residues of the NBS1 protein. In addition, immunoprecipitated ATM can phosphorylate NBS1 in vitro. (The phosphorylation is probably carried out directly by ATM protein kinase, though there could be an intervening protein kinase present in the ATM immunoprecipitates.) Several groups also showed that the phosphorylation has specific consequences. Cells expressing NBS1 mutant proteins that cannot be phosphorylated by ATM are radiosensitive and cannot delay ongoing DNA replication after damage. (This property is termed radioresistant DNA synthesis, or RDS). The failure to delay ongoing DNA replication is a checkpoint defect exhibited by both AT mutants and NBS mutants. This suggests the normal delay to DNA replication occurs, in part, via phosphorylation of NBS1 by ATM. Understanding the mechanism of S phase controls is particularly important, for failure to delay replication when DNA is damaged is likely to be a major cause of genomic instability, an important step in carcinogenesis.[12,14,15]

Possible role(s) of phosphorylation

All four reports show phosphorylation of NBS1 on two or more serine residues. The importance of multiple phosphorylation events is unclear, since each single mutation does seem to partially disrupt *NBS1* function. It is possible that phosphorylation of distinct residues could modify the R/M/N complex in different ways. For example, one phosphate modification of NBS1 could subsequently alter the endonuclease properties of MRE11 or affect ATP binding by RAD50. A second phosphate modification of NBS1 could affect localization of the complex to sites of DNA damage. While Zhao et al.[4] report a reduction in IR-induced foci formation with NBS1 double phosphorylation mutants, Wu et al. observe no change in MRE11 binding or foci distribution with NBS1 single phosphorylation mutants.[3] Alternatively, multiple phosphate modifications could play a concerted regulatory role. For example, in the MAP kinase cascade, the purpose of multiple phosphorylation sites appears to create a system of "ultrasensitivity" that has several functions, including an increase in response output of the kinases over a small change in dose of upstream signal.[16] In ultrasensitivity, the multiple phosphorylations also serve to prevent inappropriate activation of the system. Whether the R/M/N complex requires such a mode of regulation is unknown.

Two protein kinases: different forms of damage leading to common downstream pathways?

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.[4] 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.[17,18] Second, phosphorylation 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.[4,17,18]

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 disinct genes acting in common pathways seems remarkable. *P53* and *CHK2* are both associated with similar genetic diseases with dramatic predispositions 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

1. Gatei M, Young D, Cerosaletti KM, Desai-Mehta A, Spring K, Kozlov S, Lavin MF, Gatti RA, Concannon P, Khanna K. ATM-dependent phosphorylation of nibrin in response to radiation exposure. Nat Genet 2000;25:115–119.
2. Lim DS, Kim ST, Xu B, Maser RS, Lin J, Petrini JH, Kastan MB. ATM phosphorylates p95/nbs1 in an S-phase checkpoint pathway. Nature 2000;404:613–617.
3. Wu X, Ranganathan V, Weisman DS, Heine WF, Ciccone DN, O'Neill TB, Crick KE, Pierce KA, Lane WS, Rathbun G, Livingston DM, Weaver DT. ATM phosphorylation of Nijmegen breakage syndrome protein is required in a DNA damage response [see comments]. Nature 2000; 405:477–482.
4. Zhao S, Weng YC, Yuan SS, Lin YT, Hsu HC, Lin SC, Gerbino E, Song MH, Zdzienicka MZ, Gatti RA, Shay JW, Ziv Y, Shiloh Y, Lee EY.

Functional link between ataxia-telangiectasia and Nijmegen breakage syndrome gene products. Nature 2000;405:473–477.
5. Lavin MF, Shiloh Y. The genetic defect in ataxia-telangiectasia. Annu Rev Immunol 1997;15:177–202.
6. Shiloh Y. Ataxia-telangiectasia and the Nijmegen breakage syndrome: related disorders but genes apart. Annu Rev Genet 1997;31: 635–662.
7. Taylor AM, Metcalfe JA, Thick J, Mak YF. Leukemia and lymphoma in ataxia telangiectasia. Blood 1996;87:423–438.
8. Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, Tagle DA, Smith S, Uziel T, Sfez S, et al. A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science 1995;268: 1749–1753.
9. Varon R, Vissinga C, Platzer M, Cerosaletti KM, Chrzanowska KH, Saar K, Beckmann G, Seemanova E, Cooper PR, Nowak NJ, et al. Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. Cell 1998;93:467–476.
10. Carney JP, Maser RS, Olivares H, Davis EM, Le Beau M, Yates JR, 3rd, Hays L, Morgan WF, Petrini JH. The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. Cell 1998;93:477–486.
11. Stewart GS, Maser RS, Stankovic T, Bressan DA, Kaplan MI, Jaspers NG, Raams A, Byrd PJ, Petrini JH, Taylor AM. The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. Cell 1999;99:577–587.
12. Elledge SJ. Cell cycle checkpoints: preventing an identity crisis. Science 1996;274:1664–1672.
13. Weinert T. DNA damage checkpoints update: getting molecular. Curr Opin Genet Dev 1998;8:185–193.
14. Hartwell LH, Kastan MB. Cell cycle control and cancer. Science 1994; 266:1821–1828.
15. Schmutte C, Fishel R. Genomic instability: first step to carcinogenesis. Anticancer Res 1999;19:4665–4696.
16. Huang CY, Ferrell JE, Jr. Ultrasensitivity in the mitogen-activated protein kinase cascade. Proc Natl Acad Sci USA 1996;93:10078–10083.
17. Chaturvedi P, Eng WK, Zhu Y, Mattern MR, Mishra R, Hurle MR, Zhang X, Annan RS, Lu Q, Faucette LF, et al. Mammalian Chk2 is a downstream effector of the ATM-dependent DNA damage checkpoint pathway. Oncogene 1999;18:4047–4054.
18. Siliciano JD, Canman CE, Taya Y, Sakaguchi K, Appella E, Kastan MB. DNA damage induces phosphorylation of the amino terminus of p53. Genes Dev 1997;11:3471–3481.
19. Bentley NJ, Holtzman DA, Flaggs G, Keegan KS, DeMaggio A, Ford JC, Hoekstra M, Carr AM. The Schizosaccharomyces pombe rad3 checkpoint gene. EMBO J 1996;15:6641–5651.
20. Cliby WA, Roberts CJ, Cimprich KA, Stringer CM, Lamb JR, Schreiber SL, Friend SH. Overexpression of a kinase-inactive ATR protein causes sensitivity to DNA-damaging agents and defects in cell cycle checkpoints. EMBO J 1998;17:159–169.
21. Brown EJ, Baltimore D. ATR disruption leads to chromosomal fragmentation and early embryonic lethality. Genes Dev 2000;14:397–402.
22. Carr AM. Control of cell cycle arrest by the Mec1sc/Rad3sp DNA structure checkpoint pathway. Curr Opin Genet Dev 1997;7:93–98.
23. Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DC, Shannon KE, Lubratovich M, Verselis SJ, Isselbacher KJ, Fraumeni JF, et al. Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome [see comments]. Science 1999;286:2528–2531.
24. Malkin D, Li FP, Strong LC, Fraumeni JF, Jr, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 1990;250:1233–1238.
25. Cortez D, Wang Y, Qin J, Elledge SJ. Requirement of ATM-dependent phosphorylation of brca1 in the DNA damage response to double-strand breaks. Science 1999;286:1162–1166.
26. Gowen LC, Avrutskaya AV, Latour AM, Koller BH, Leadon SA. BRCA1 required for transcription-coupled repair of oxidative DNA damage. Science 1998;281:1009–1012.
27. Lee H, Trainer AH, Friedman LS, Thistlethwaite FC, Evans MJ, Ponder BA, Venkitaraman AR. Mitotic checkpoint inactivation fosters transformation in cells lacking the breast cancer susceptibility gene, Brca2. Mol Cell 1999;4:1–10.
28. Scully R, Chen J, Plug A, Xiao Y, Weaver D, Feunteun J, Ashley T, Livingston DM. Association of BRCA1 with Rad51 in mitotic and meiotic cells. Cell 1997;88:265–275.
29. Wu X, Petrini JHJ, Heine WF, Weaver DT, Livingston DM, Chen J, Zhong Q, Chen, C-F, Chen P-L, Lee W-H. Independence of R/M/N focus formation and the presence of intact BRCA1. http://www.sciencemag.org/cgi/content/full/289/5476/11a.
30. Wang Y, Cortez D, Yazdi P, Neff N, Elledge SJ, Qin J. BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. Genes Dev 2000;14:927–939.
31. Rotman G, Shiloh Y. Ataxia-telangiectasia: is ATM a sensor of oxidative damage and stress? Bioessays 1997;19:911–917.
32. Lee JS, Collins KM, Brown AL, Lee CH, Chung JH. hCds1-mediated phosphorylation of BRCA1 regulates the DNA damage response. Nature 2000;404:201–204.