Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

Kurt W. Kohn, MD, PhD
Scientist Emeritus
Laboratory of Molecular Pharmacology
Developmental Therapeutics Branch
National Cancer Institute
Bethesda, Maryland
kohnk@nih.gov

CHAPTER 29

The ataxia telangiectasia story and the ATM gene.

In 1941 Madame Denise Louis-Bar, at the time a resident in the neurological clinic of the Bunge Institute at Antwerp, Belgium, saw an unusual case of a 9-year-old boy, who had a combination of neurological and blood vessel symptoms. On the neurological side, the child had difficulty walking, discoordination of movements and slurred speech ("ataxia"), which she attributed to a problem in the cerebellum. There were several known diseases with ataxia. However, the child also had clusters of enlarged small blood vessels ("telangiectasia") in several places, such as the eyes, face, and ears. She thought this combination of symptoms was unusual and wrote up the case in great detail in an article entitled "*Sur un syndrome progressif comprenant des telangiectasies capillaires cutanees et conjonctivales symmetriques a disposition naevoide et des troubles cerebelleux*," which was published in *Confinia neurol. 4:32, 1941* (Boder and Sedgwick, 1958). I could not find the original 1941 article, nor a picture of Madame Louis-Bar. However, her paper became a landmark, and for a time the disease was called "Louis-Bar syndrome."

However, there was an earlier report, in 1926, by two Czech neurologists of 3 siblings with this syndrome (Henner, 1968; Syllaba and Henner, 1926) (Figure 29.1). These children's ataxia was in the form of "chorea-athetosis" (involuntary and writhing movements) and they had telangiectasias, but no pulmonary infections. As their family's economic situation deteriorated and the siblings' disability became unmanageable, all 3 of them eventually resorted to suicide (Etzioni et al., 2014).

Between 1950 and 1957, eight children were seen with this combination of progressive ataxia of the cerebellar type beginning in early childhood together with striking telangiectasia in the eyes and in areas of the upper face ("butterfly area"). In addition, many of them had recurrent infection of the lungs, sinuses, and middle ear. Often, there were other family members with similar symptoms. This pattern of symptoms was noted by Elena Boder and Robert P. Sedgwick of the University of Southern California, Los Angeles

Children's Hospital, and Cedars of Lebanon Hospital, who concluded that it was a syndrome, which they named "ataxia-telangiectasia." In 1958, these authors published an extensively detailed report of these cases (Boder and Sedgwick, 1958) (Figure 29.2). But they were still unaware of the 1941 paper by Madame Louis-Bar, until it was brought to their attention by Professor Ludo van Bogaert of the Bunge Institute, who had in fact cared for the patient described by Madame Louis-Bar. However, none of the aforementioned were aware of the even earlier report by Syllaba and Henner (Syllaba and Henner, 1926).

Typically, the disorder was first noted when the 2-year-old began to walk and seemed unsteady and clumsy, and the unsteady gait became worse with time. Dilated blood vessels (telangiectasia) in the eyes and over the bridge of the nose typically appeared at the age of 4 or 5. One of the 8 children in the original report was noted to have some grey hair at the age of 2. The children began to speak at the usual time and their intelligence was near normal, but their speech became slurred and eventually it became hard to understand what they were saying. In addition, they had tremor and increasingly discoordinated movements typical for pathology of the cerebellum. As they grew older, the children were much below average height and weight for their age.

I was especially moved by the picture of one of their patients, a 9-year-old girl (Figure 29.3). She was a typical case of this disease, which I will summarize below. She had an older sister with this same rare disease; it is hard to imagine the anguish of a family with even one child with this slowly fatal disease.

Figure 29.4 shows a close-up of her eye that shows the dilated blood vessels in the corner of the eye and bridge of her nose (telangiectasias). These areas are typically affected in ataxia-telangiectasia. They are areas that could be exposed to sunlight and cause DNA damage that could not be repaired because of a gene mutation in a DNA repair gene characteristic of the disease (to be explained later in this chapter).

Occasionally, an older child with this syndrome was found to have a malignant lymphoma or sarcoma. There was sometimes autopsy evidence of premature aging, such as early atherosclerosis and arthritis.

Since the ataxia symptoms were of the kind produced by pathology of the cerebellum, special attention was given to that part of the brain in patients who came to autopsy. Although the outward appearance of the cerebellum seemed normal, microscopic examination showed marked degeneration of certain cell types, especially the large Purkinje cells (Figures 29.6 and 7). There will be much to say about how this connects up with the other symptoms of ataxia telangiectasia so as to make a coherent story.

But first, what are Purkinje cells? They are large cells arranged side-by-side in a layer in the cerebellum. Coming out of each of them towards the surface of the layer is a large bush of dendrites. Johanne Evangelista Purkinje (Figure 29.8) discovered these cells in 1839, and Santiago Ramon y Cajal developed a stain that clearly showed their amazing axon bushes (Figure 29.6). The Purkinje cell layer is the output funnel for all of the input from the rest of the cerebellum. Their extensive bush of dendrite branches allows them to assemble inputs from a great many other neurons. There is a big burden on the Purkinje cells, however, because they

have to fire rapidly to keep up with rapid changes in balance and coordinated movements, including the remarkable feats of athletes and circus performers.

To carry out their tasks during rapid movements, the Purkinje cells need a large continual supply of energy. The energy is generated in the cells' mitochondria, which produce oxidation products as a side-reaction that can damage DNA. The cells have powerful antioxidants that neutralize most of these molecules, but inevitably some escape and attack the DNA. The cells have mechanisms to repair the DNA damage, and these mechanisms are critically important to prevent the accumulation of DNA damage. We will see that ataxia telangiectasia is due to an inherited mutation in a DNA repair gene called *ATM*; the ataxia may be due to this DNA repair defect having a particularly strong impact on Purkinje cells (Baltanas et al., 2011a; Baltanas et al., 2011b).

By 1964, a research team led by Robert A. Good at the University of Minnesota ("the Good guys") showed that ataxia telangiectasia is a primary immunodeficiency disease, which accounts for the frequent infections that were the most frequent cause of death (Peterson et al., 1964) (Etzioni et al., 2014). This however did not explain the ataxia or the telangiectasia.

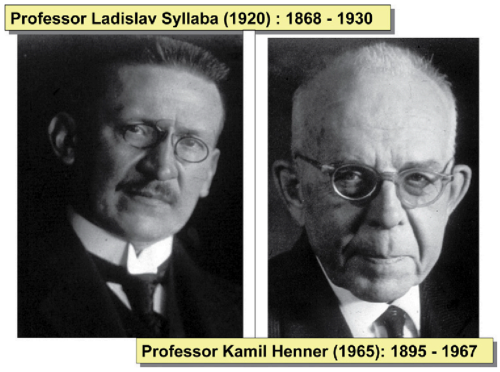

Figure 29.1. Czech Professors Ladislav Syllaba and Kamil Henner, the first to describe patients with the inherited disease that came to be called ataxia telangiectasia (picture from (Etzioni et al., 2014).

Figure 29.2. Elena Boder and Robert P. Sedgwick definitively described 8 cases of ataxia-telangiectasia in 1958 (picture from (Etzioni et al., 2014)).

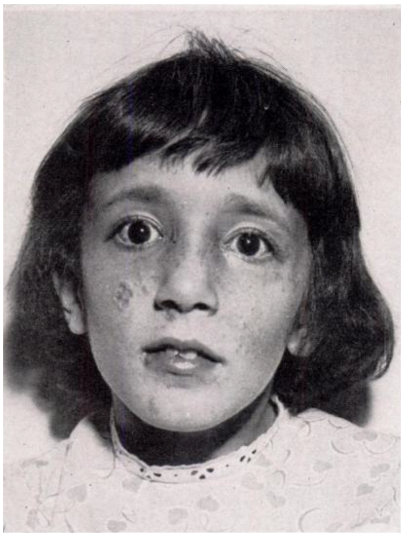

Figure 29.3. A 9-year old girl with ataxia-telangiectasia (Boder and Sedgwick's case 1) (Boder and Sedgwick, 1958). She was unabIe to walk and hardly able to speak or eat, but her mind was clear; she died 2 years after this picture was taken. I like to think she may sometimes have escaped her distress by living in an imaginative world of adventure, maybe like that depicted by Maurice Sendak in "Where the Wild Things Are" 1963, Harper Collins Publishers.

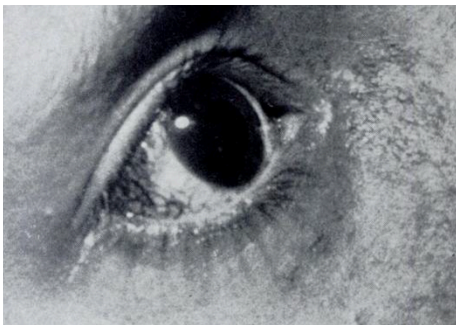

Figure 29.4. Closeup of her right eye, showing enlarged blood vessels in the corner of the eye (which might be where exposure to sunlight could occur). Telangiectasia is also seen along the bridge of the nose, which is also an area subject to sunlight exposure (right side of the picture).

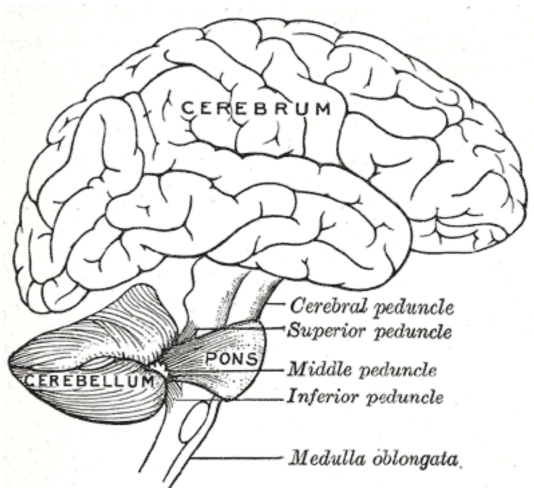

Figure 29.5. The cerebellum in relation to other parts of the brain. Although smaller than the cerebrum, it contains many more neurons. In contrast to the convoluted gyri of the cerebral cortex, the cerebellum is formed of closely spaced deep grooves that provide a large surface area for a layer of neurons giving an enormous computation power to coordinate body movements. (From Gray's Anatomy, 1918.)

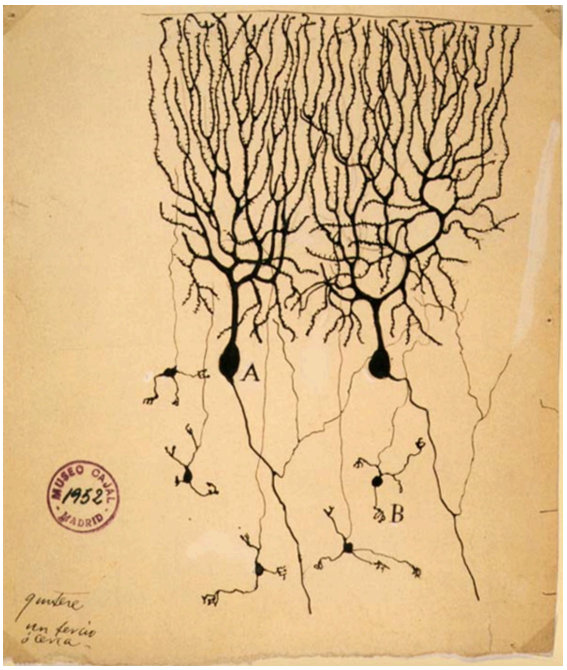

Figure 29.6. Purkinje cells of the cerebellum. These large cells are lined up in a layer; they have the most extensive interlocking system of branches in the nervous system. This drawing was made by the famous cytologist Santiago Ramon y Cajal in 1899; *Instituto Cajal*, Madrid, Spain. It shows two Purkinje cells (A) and connections from the smaller granular cells (B). In ataxia-telangiectasia, both of these cell types are decimated. (From Wikipedia.)

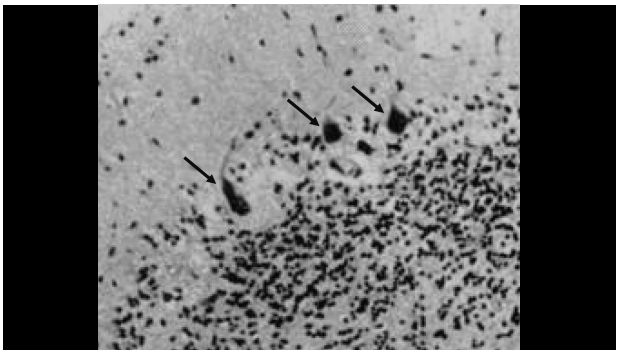

Figure 29.7. Section through the patient's cerebellum showing Purkinje cells (arrows): they are few and far between, whereas normally there would be many more lined up side-by-side in a layer (from case 1 of Boder et al. 1958 (Boder and Sedgwick, 1958), arrows added). The paucity of these cells explains her inability to carry out controlled movements, but the loss of these and other cells of the cerebellum did not impair her mind.

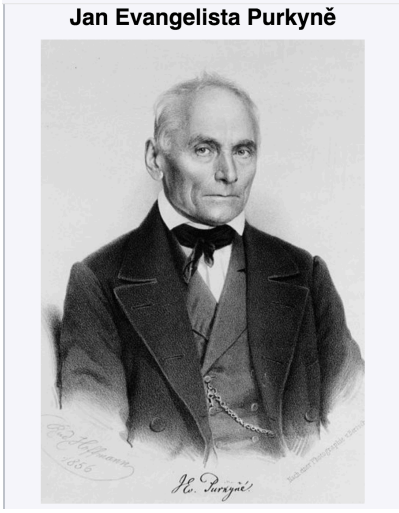

Figure 29.8. Johanne Evangelista Purkinje (1787-1869), Czech anatomist who discovered the Purkinje cells of the cerebellum. He had graduated from University of Prague and worked at University of Breslau. (From Wikipedia).

The gene responsible for ataxia telangiectasia identified at last.

The next step to locate and clone the gene responsible for the ataxia telangiectasia disease was not undertaken until the 1980's, after the required technology was developed. It was nevertheless a complicated task consuming the efforts of many researchers in several laboratories (Etzioni et al., 2014; Savitsky et al., 1995). The first breakthrough along the way to this accomplishment, reported in 1988 by researchers at UCLA School of Medicine Los Angeles and their collaborators, applied a genetic method called linkage analysis that localized the genome region responsible for the disease to the q22-23 region of chromosome 11 (Gatti et al., 1988). Mutations in this region seemed to be responsible for most cases of the disease. With the new methods of molecular genetics, a large group of researchers, led by Yosef Shiloh, were then able to isolate and sequence the DNA of a large part of the responsible gene, which they dubbed *ATM* for ataxia telangiectasia mutated (Savitsky et al., 1995). In this way, the name of the disease evolved from Louis-Barr syndrome to ataxia telangiectasia to ATM disease.

By 1963, more than 100 cases of ataxia-telangiectasia had been identified and shown that the disease was inherited as a single mutated gene with an autosomal recessive trait, meaning that the disease occurred only when the person had a mutated *ATM* gene in both chromosomes 11, having inherited one from the father and one from the mother. Each parent would have had one chromosome 11 with a mutated gene and one with the normal gene, and the unfortunate child would have inherited both defective chromosomes.

Those "haploid" individuals (about 1% of the population) who had one chromosome 11 with and one without the mutation were carriers of the ATM disease. They were disease-free but had increased sensitivity to radiation and an increased cancer risk, although their

risk was much less than in ataxia telangiectasia patients, for whom a cancer of the lymphatic system was the second most frequent cause of death (Etzioni et al., 2014). People who carried one copy of the *ATM* gene were estimated to have a three or fourfold increased risk of developing cancer, although the increased risk of breast cancer in women was about fivefold and tended to occur at an earlier age (Etzioni et al., 2014; Savitsky et al., 1995).

How ATM is activated.

In 2003, Christopher Bakkenist and Michael Kastan at St. Jude Children's Hospital in Memphis, Tennessee investigated the phosphorylation of ATM and its consequences in response to DNA damage (Bakkenist and Kastan, 2003). They found that ATM exists as an inactive homodimer that responds to x-ray-induced DNA damage with each ATM unit phosphorylating its partner at serine-1981. Over 50% of the cell's ATM became autophosphorylated within a few minutes after the irradiation. The authors felt that there were too few DNA damage sites for direct action at those sites solely to account for the rapid phosphorylation of such a high fraction of the ATM. The resulting ATM monomers were active protein kinases. The activated ATM then phosphorylated several proteins that had been found to block or delay cell cycle at a checkpoint. These included, among others, p53 and Chk2 that blocked the cells at the G1/S checkpoint; Nbs1 and Brca1 that delayed S phase; Brca1 also blocked the cells at the G2/M checkpoint. Cells from ataxia-telangiectasia patients, both of whose ATM genes were mutated, repaired DNA strand breaks normally; the defect in the cell's DNA damage response was instead thought to be in the cell cycle checkpoints.

The many actions of ATM in response to DNA damage.

Ataxia telangiectasia (where both chromosomes 11 had a mutated ATM gene) was found to have chromosome instability, meaning that the patients' cells often had an abnormally high frequency of chromosome breakage both with and without DNA damage, induced for example by x-rays (Lehmann and Carr, 1995). The patients were often extraordinarily sensitive to x-rays -- which was first noted when some died even after having an ordinary diagnostic x-ray. The high x-ray sensitivity was noted also in their cells, which were killed by unusually low doses of x-rays or by DNA damaging anticancer drugs. However, the ataxia telangiectasia cells seemed to be able to repair DNA strand breaks normally (Taylor et al., 1975), in contrast to cells from xeroderma pigmentosum patients, which had greatly reduced ability to repair DNA strand breaks (Chapter 22). Evidently, the ataxia telangiectasia genetic defect was not simply in the DNA repair machinery itself.

In 1980, Bob Painter at the University of California, San Francisco discovered the main defect in ataxia telangiectasia cells: the cells were defective in responding to damaged DNA by slowing their rate of DNA replication (Painter and Young, 1980) (Figure 29.9). The decreased DNA synthesis rate helped normal cells survive DNA damage by giving the cells

more time to repair their damaged DNA before initiating mitosis, when persistent DNA damage would produce unrepairable chromosome damage. It was as if the ataxia telangiectasia cells were unable to detect or respond properly to DNA damage. This was the first evidence for a "checkpoint" at which cells checked whether it was safe to proceed along the cell division cycle (Lehmann and Carr, 1995).

This kind of "checkpoint" response to DNA damage was found in a wide variety of organisms. Two major checkpoints were at two points in the cell division cycle. The first was at the point in time when the cell begins to replicate its DNA; this checkpoint response (known as the G1-S checkpoint) would delay the onset of DNA replication. The second (known as the G2-M checkpoint) was at the point in time when the cell begins to condense its chromosomes in preparation for mitosis; this checkpoint would delay the preparation for mitosis. Both checkpoints served to give more time for DNA repair before the cell begins a process during which the presence of DNA damage would likely damage or possibly kill the cell.

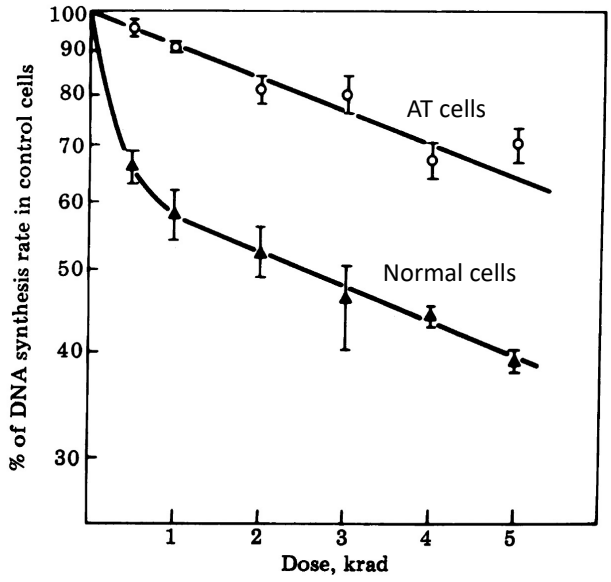

Figure 29.9. Normal cells (lower curve) responded to x-rays by decreasing their rate of DNA synthesis. Ataxia telangiectasia cells (upper curve) were largely defective in their ability to do that (Painter and Young, 1980). Horizontal axis: radiation dose in thousands of rad. Vertical axis: % of DNA synthesis compared to unirradiated cells.

How the ATM gene and protein control the cell cycle checkpoint began to be elucidated in 1992 by researchers led by Michael Kastan at Johns Hopkins Oncology Center in Baltimore, Maryland and by a group led by Albert Fornace in my Laboratory at NIH (Kastan et al., 1992). They found that inducing DNA damage by x-rays in cells from ATM patients did not cause the usual increase the level of p53 protein seen in normal cells. The function of a normal p53 protein was required for the normal cell cycle checkpoint response to DNA damage. (p53 will be the subject of a later Chapter.) This indicated that the checkpoint response required an action of a normal ATM protein on a normal p53 protein: there was a

pathway from DNA damage to ATM to p53 to checkpoint response. Knocking out p53 prevented the response; there was no response if ATM was mutated (the cells were from an ATM patient) or if the cells' p53 was inactivated by mutations. They also found another factor in the pathway, a protein called GADD45 whose gene was discovered and characterized by Al Fornace in my Lab. DNA damage caused GADD45 to bind tightly to p53, and this did not happen in ATM cells. In the absence of GADD45, there was no checkpoint response to DNA damage. Thus, there was a pathway: from DNA damage to ATM to p53 to GADD45 to checkpoint response.

ATM's molecular functions were further investigated in Michael Kastan's laboratory at St. Jude Children's Research Hospital in Memphis, Tennessee (Bakkenist and Kastan, 2003; Berkovich et al., 2007). They found that ATM normally exists as an inactive dimer. In response to DNA damage, the two ATM molecules of the dimer phosphorylate each other, thereby causing the dimer to separate to form active monomer units able to phosphorylate the hydroxyls of serine or threonine in several proteins. This dimer separation and activation was somehow brought about through the action of a remarkable trimer that we encountered in Chapter 27B as an agent that detects DNA damage and initiates its repair. The three parts of the trimer were Mre11, Rad50, and Nbs1. The trimer, abbreviated MRN, here again detects the DNA damage and induces the inactive ATM dimer to separate and use its kinase (i.e., phosphorylating) activity

The relationship between ATM and MRN turned out to be quite deep. To begin with, ataxia telangiectasia (ATM disease) was found to be closely related to Nijmegen breakage syndrome (NBS) (described in Chapter 27A), both in the symptoms of the diseases and in the functional relationships of their respective genes (Shiloh, 1997). The two diseases shared high sensitivity to radiation, high incidence of cancer, chromosome instability and deficiency of the immune system, and they both lacked the checkpoint responses to DNA damage that delayed the progress of normal cells through the cell cycle. The only major difference was that NBS patients lacked the cerebellar deterioration that caused ataxia in ATM disease. The most frequent cancers of patients with ATM disease were lymphomas and lymphoid leukemias of both the B-cell and T-cell type, which were hundreds of times more frequent than in the general population (Shiloh, 1997).

ATM was found to have amazingly many actions in response to DNA damage and coordinating the repair. The gene is large and located in chromosome 11. The domain structure of the protein, summarized in Figure 29.10A, closely resembled the ATM-related ATR protein as well as the DNAPKcs that was a major player in the repair of DNA double-stand breaks (DSB) (Chapter 27B). A more detailed function diagram of ATM's domains showed the many interactions of this remarkable protein (Figure 29.10B) (Phan and Rezaeian, 2021). Particularly notable was the interaction with Nbs1, a component of the Mre11-Rad50-Nbs1 (MRN) complex that signals the presence of DNA damage response elements such as delayed mitosis to allow time for DNA repair (Figure 29.11 and Chapter 27A, Figure 27A.13).

Figure 29.11 summarizes the actions of ATM: By way of MRN and BRCA1, ATM stimulates repair of DNA double-strand breaks. By phosphorylating the checkpoint kinase Chk2,

which then phosphorylates and inactivates the phosphatase CDC25A, ATM inhibits progress of the cell cycle. In response to DNA damage, ATM phosphorylates and activates p53, which then activates the transcription of p21/CDKN1A that inhibits the cyclin-dependent-kinase Cdk2 -- which blocks the cell cycle in G1. P53 also stimulates the transcription of the apoptosis initiators Bax, Bid, and PUMA, causing cells to die by suicide. This is but an outline of the central role of ATM in the cell's complex responses to DNA damage, as currently understood (Phan and Rezaeian, 2021).

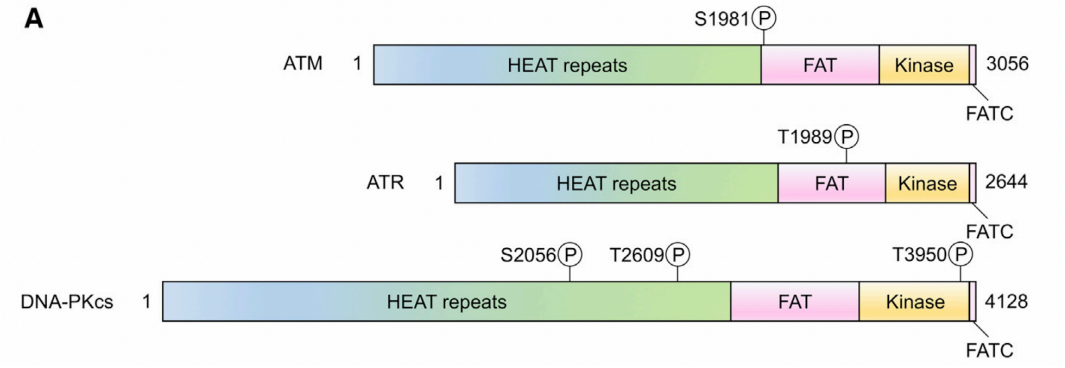

Figure 29.10A. Similarity of ATM's domain structure to that of the functionally related ATR and DNAPKcs proteins (Blackford and Jackson, 2017). All three proteins have a serine/threonine kinase domain in the C-terminal regions. They have a so-called FAT domain that serves to bind proteins that the kinase domain phosphorylates. The large HEAT repeat region has multiple functions. It contains repeats, consisting of two helices separated by a short loop, that form a kind of solenoid structure.

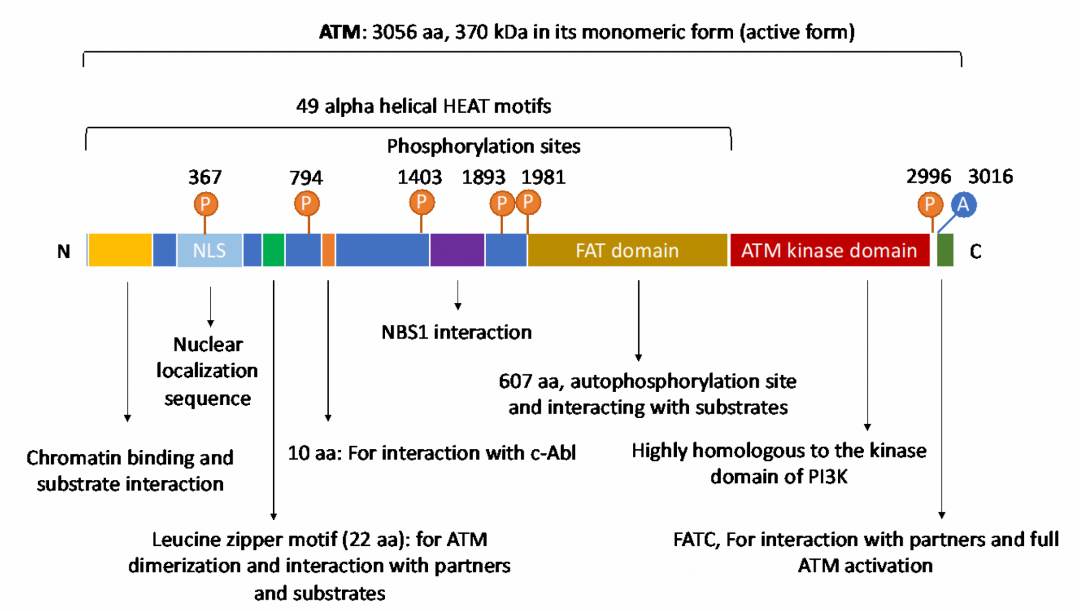

Figure 29.10B. A more detailed diagram of the many functions of the various parts of the human ATM protein (Phan and Rezaeian, 2021). By way of its N-terminal region, the

protein binds to chromatin and to DNA damage response proteins, such a p53. Nearby, there is a nuclear localization sequence that enables the protein to enter the nucleus. The "leucine zipper" motif keeps the protein in an inactive homodimer state until a phosphorylation event serves to activate it. Then there are sites for interaction with ABL (see Chapter 14) and NBS (see Chapter 27A). Several of the phosphorylations, as well as the acetylation site at position 3016 at the C-terminus, serve to activate ATM's kinase function.

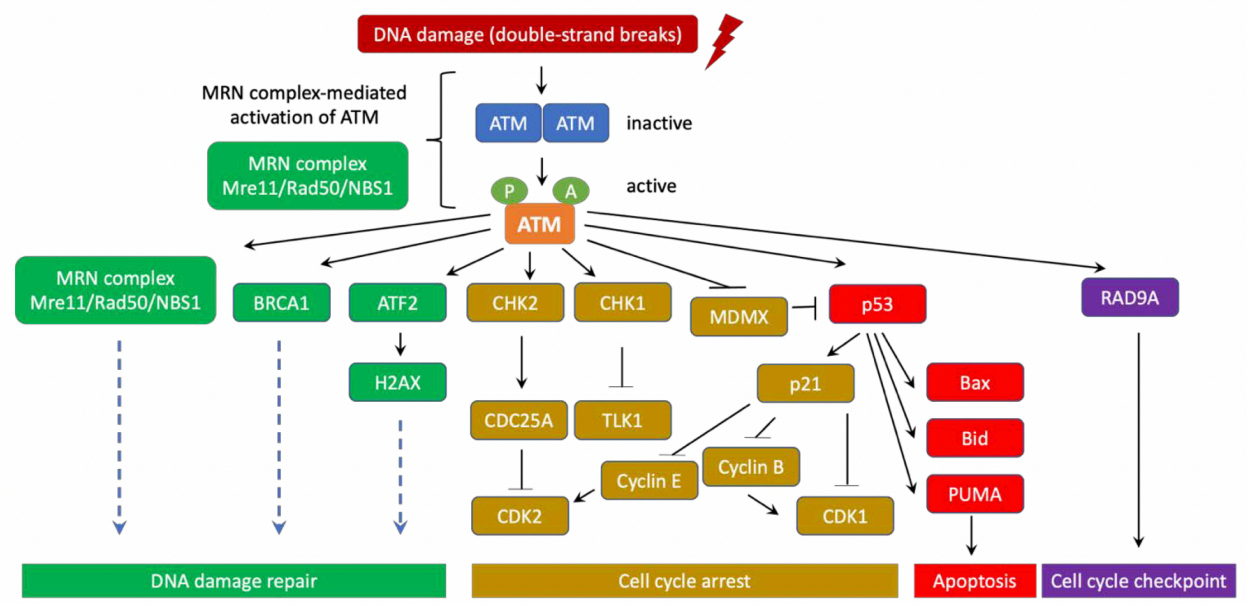

Figure 29.11. The multiplicity of the actions of ATM in response to DNA double-strand breaks (DSB). ATM helps DNA repair and signals cell cycle delay and, as a last resort, cell suicide by apoptosis (Phan and Rezaeian, 2021).

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