Chapter 28. The gammaH2AX story 221009dh3.docx

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

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CHAPTER 28

The gamma-H2AX story: DNA double-strand breaks revealed in cell nuclei.

In 1976, my Laboratory was fortunate to be joined by William M. Bonner, who had become interested in histones during his post-doctoral work at Oxford and Cambridge in England (Figure 28.1). Bill was one of those senior researchers who did much of the lab work himself. Thus, he had a hands-on feel for the experiments that, perhaps much to his own surprise, yielded ground-breaking advances for investigations of DNA damage repair and cancer.

Our Laboratory had begun to study histones in the late 1960's, because these proteins were intimately associated with the chromatin where DNA damage and repair were taking place. But in those early days, we were still abysmally ignorant about histones and chromatin: the identity of the histone proteins was still hazy, and we did not yet know about nucleosomes! When Bill joined our laboratory, the nucleosome structure of chromatin had already come to light and the histone proteins had been well defined. Bill had a new and better understanding of chromatin structure and began in his modest lab to study the individual histone proteins carefully and systematically. He proceeded by carefully pinning down one step after another, without speculating too far into the future. I think he had no idea that his work would lead to something so important.

His initial objective was to improve the gel electrophoresis method to increase its ability to separate the different histones and their sub-types. That would enable him to investigate how each of those sub-types would respond after cells were treated in different ways. He devised a new 2-dimensional gel system in which the histones could be separated, first by electrophoresis in one direction, followed by electrophoresis in the perpendicular direction in a different medium (e.g., acetic acid-urea). (gel electrophoresis separates histones, largely based on their positive charge, which causes them to move in an electric field.)

His new electrophoresis method revealed histone variant proteins that had never been seen before. He found the variants of histone H2A particularly amenable for study, because they were seen as distinctly separated spots in his gels. He had no idea what proteins were in those spots, so he called them H2AX and H2AZ. I don't know what happened to H2AY, but the other two had very bright futures as major players in the cell. Bill investigated both of them but focused mostly on H2AX – and this led to one of the most far-reaching discoveries in the field of DNA damage repair. Ironically, his early work on histone proteins had no obvious direct bearing on cancer research, but I supported it, because the histones surely had a role in most DNA functions, including what happens when anticancer drugs attack DNA. The irony was that, although we had no idea how his work would be relevant to that specific focus of our Laboratory, his contribution was one of the most important advances to come from our Laboratory – and it turned out to be directly relevant to our focus on anticancer drugs that attack DNA!

When Bill Bonner modified the electrophoresis conditions to further increase its resolution, three modified forms of H2AX were revealed, which he marked alpha, beta, and gamma. A remarkable observation was that the amount of gamma-H2AX increased when the cells were exposed to radiation that damaged their DNA: gamma-H2AX became an exquisitely sensitive measure of the amount of DNA damage in the cells (Rogakou et al., 1998) (Figure 28.2 and 28.3).

Importantly, they found that the gamma-H2AX that appeared after radiation was phosphorylated at a single position, namely at amino acid number 139, which is a serine near the C-terminal end of the protein's amino acid chain. A question that then arose immediately, of course, was, what is the enzyme that brings about that phosphorylation and what might be its role in the response of cells to DNA damage. It took much research to answer these questions, and the answer had important bearing on the cell's response to anticancer drugs, as we shall see.

What they did soon find out, however, was that the kind of DNA damage to which gamma-H2AX responded was double-strand breaks (DSB). The response developed within a mere few minutes, during which a large number of gamma-H2AX molecules clustered around each double-strand break. Each of those clusters became visible as "foci": fluorescent spots in the cell nucleus, made visible by means of a fluorescent antibody (Figure 28.4) (Bonner et al., 2008). The amount of radiation damage existing in a cell could be gaged by counting the number of foci in its nucleus, which was equivalent to counting the number of DNA double-strand breaks! More practical was to measure the intensity of the fluorescence produced by the gamma-H2AX antibody in a cell or tissue sample.

The repair of the DNA double-strand breaks (DSB) could be seen as the gamma-H2AX foci disappeared. The appearance and disappearance of these foci became a powerful tool for studying the effects of anticancer drugs and the cell's response. Also, it turned out that other proteins clustered together, some within the gamma-H2AX foci and some in foci of other kinds. Later, we will discuss some of those proteins and their bearing on how proteins in the cell signal the presence of DNA damage and protect the cell by inhibiting the cell division cycle or kill the cell by inducing programmed cell death (apoptosis).

Bonner's group also found that, co-localized with those same gamma-H2AX foci, were the DNA repair proteins Rad50, BRCA1, and Rad51 (Rogakou et al., 1998). (Figure 28.5). The DNA repair functions of these proteins was already discussed in the precious two chapters. Clearly and remarkably, therefore, a set of DNA repair proteins clustered around each DNA double-strand break, consistent with the DSB repair models discussed in those chapters. The different repair proteins, however, did not appear in the foci all at the same time. The gamma-H2AX foci appeared first, shortly after the cells were irradiated. Brca1 then gradually appeared in the foci, followed by Rad50 (Paull et al., 2000), which exists in a complex of three proteins: Mre11:Rad50:Nbs1 (MRN complex), whose function was discuss later in the previous chapter.

So, what did this method reveal when applied to anticancer drugs?

In cells treated with camptothecin, DNA double-strand breaks appeared, but only in cells that were in S phase (Bonner et al., 2008) (Figure 28.4). This was consistent with how camptothecin perturbs DNA replication (see Chapter 11).

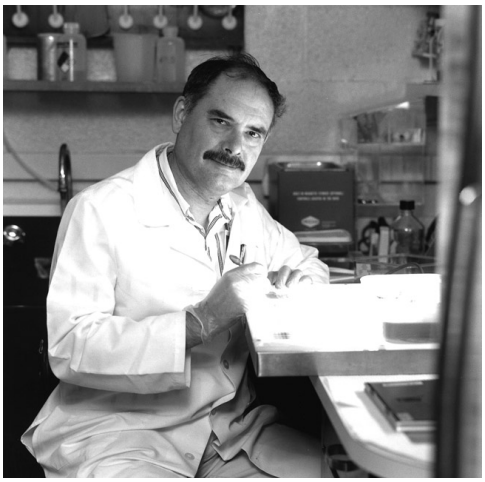

Figure 28.1. William M. Bonner in his laboratory in 1995. Bonner is noted for his landmark discovery of the histone gamma-H2Ax protein and its role in DNA damage repair. After receiving a PhD from Harvard University in Biochemistry and Molecular Biology, working with Nobel Prize winner Konrad Bloch, he carried out postdoctoral studies at Oxford University and the MRC Laboratories in Cambridge, England, where he became interested in histones. He continued this work at NIH as a Staff Fellow in the National Institute of Child Health and Human Development. Two years later, in 1976, he moved to NCI's Laboratory of Molecular Pharmacology. In 1980, he identified two important variants of histone H2A, which he named H2AX and H2AZ. (Source: National Cancer Institute; photo by Bill Branson; public domain.)

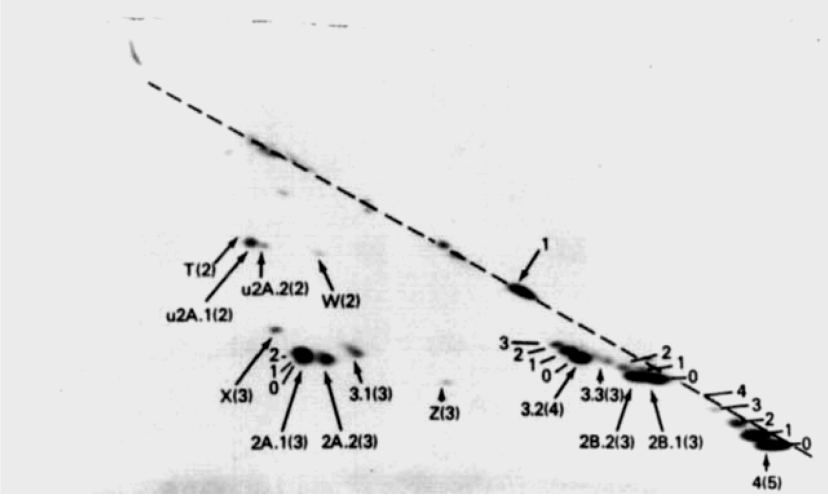

Figure 28.2. Two-dimensional gel electrophoresis, developed by William M. Bonner in my laboratory, showing the separation of histone variant proteins (spots below the diagonal). The protein in the spot labeled X(3) was to become known as H2AX (Bonner et al., 1980).

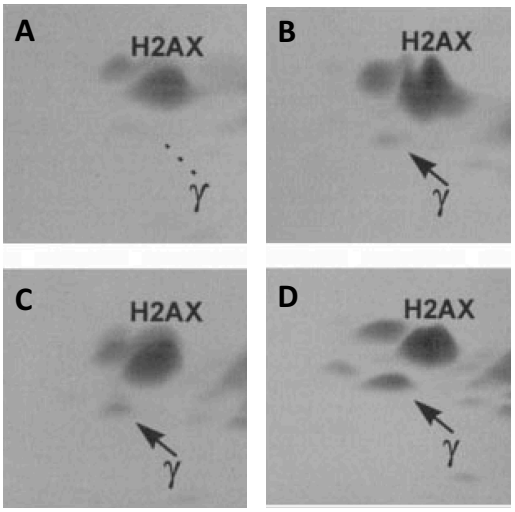

Figure 28.3. Higher resolution gels disclosed three variants in addition to the main H2AX spot. The variant labelled gamma (arrow) increased when the cells had been subjected to radiation: **A**, unirradiated cells; **B-D**, cells exposed to increasing doses of radiation. Gamma-H2AX became famous as a measure of DNA double-strand breaks in the cell. *(From Rogakou, Pilch, Orr, Ivanova, and Bonner, 1998, Journal of Biological Chemistry* (Rogakou et al., 1998))

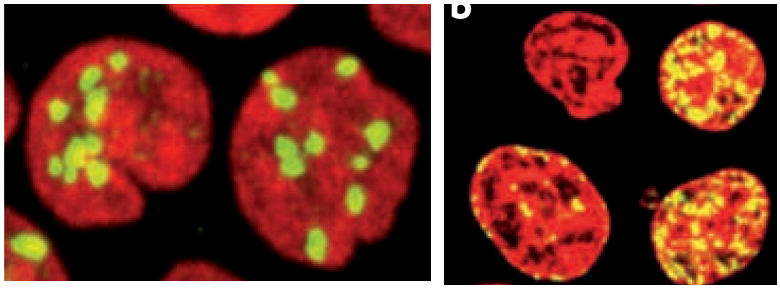

Figure 28.4. Gamma-H2AX foci in the nuclei of cells exposed to radiation (30 minutes after 1 Gy) (*left*) or to camptothecin (*right*). Radiation produced foci in all of the cells, whereas camptothecin produced foci only in cells that were in S phase. (Bonner et al., 2008).

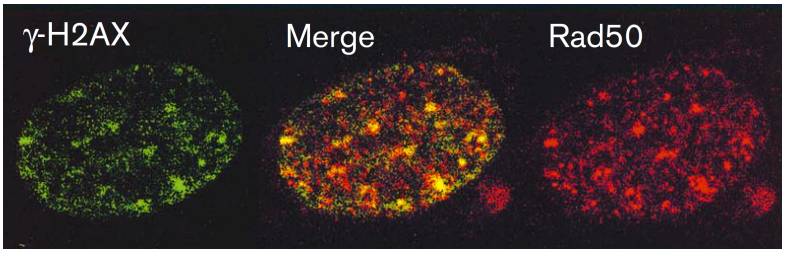

Figure 28.5. Gamma-H2AX foci in the nuclei of irradiated cells (left, green); each focus is a cluster of gamma-H2AX proteins at a DNA double-strand break. Another DNA repair protein, Rad50, also clusters in foci (right, red), and co-localizes with the gamma-H2AX foci, as can be seen in the merged image (center), where the coinciding green and red are seen as white. Therefore, gamma-H2AX and Rad50 clustered together around each DNA double-strand break. Nine double-strand breaks are evident in this cell nucleus (white spots in the merged image). Similar experiments showed that Brca1 and Rad51 also cluster together in those same foci. (Gamma-H2AX was labeled with a green fluorescent antibody, and Rad50 was labeled with a red fluorescent antibody.) (Rogakou et al., 1998).)

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