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Characteristics of γ-H2AX foci at DNA double-strand breaks sites

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Abstract: Phosphorylated H2AX (γ-H2AX) is essential to the efficient recognition and (or) repair of DNA double strand breaks (DSBs), and many molecules, often thousands, of H2AX become rapidly phosphorylated at the site of each nascent DSB. An antibody to γ-H2AX reveals that this highly amplified process generates nuclear foci. The phosphorylation site is a serine four residues from the C-terminus which has been evolutionarily conserved in organisms from giardia intestinalis to humans. Mice and yeast lacking the conserved serine residue demonstrate a variety of defects in DNA DSB processing. $H2AX^{\Delta/\Delta}$ mice are smaller, sensitive to ionizing radiation, defective in class switch recombination and spermatogenesis while cells from the mice demonstrate substantially increased numbers of genomic defects. γ-H2AX foci formation is a sensitive biological dosimeter and presents new and exciting opportunities to understand important biological processes, human diseases, and individual variations in radiation sensitivity. These potentialities demonstrate the importance of understanding the parameters and functions of γ-H2AX formation.

Résumé : L'histone H2AX phosphorylée (H2AX γ) est essentielle pour la reconnaissance et/ou la réparation efficace des cassures double brin de l'ADN. De nombreuses molécules (souvent des milliers) de H2AX sont rapidement phosphorylées au site de chaque cassure double brin dès qu'elle se produit. Un anticorps dirigé contre l'histone H2AX γ révèle que ce processus très amplifié entraîne la formation de foyers nucléaires. Le site de phosphorylation est le 4^{e} résidu de l'extrémité C-terminale, une sérine conservée au cours de l'évolution chez les organismes allant de *Giarda intestinalis* aux humains. Il y a diverses anomalies de la réparation des cassures double brin de l'ADN chez les souris et la levure comportant pas ce résidu sérine. Les souris $H2AX^{\Delta/\Delta}$ sont plus petites; elles sont sensibles aux radiations ionisantes et ont des anomalies de la recombinaison de commutation de classe et de la spermatogenèse. Les cellules de ces souris ont un nombre nettement augmenté d'anomalies génomiques. La formation des foyers de H2AX γ constitue un dosimètre biologique sensible et nous donne de nouvelles occasions excitantes de comprendre des processus biologiques importants, des maladies humaines et les variations individuelles de la sensibilité aux radiations. Cela montre l'importance de comprendre comment et pourquoi il y a formation de H2AX γ.

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Introduction

DNA double strand breaks (DSBs) are one of the most serious DNA lesions compromising genomic integrity (Rothkamm and Lobrich 2002; Jackson 2002). Since both strands of the double helix are broken the coding redundancy of the Watson and Crick strands is lost. Repair of such DSBs may be largely error-free by homologous combination with a sister chromatid or the homologous chromosome; however, the lesions may also be rejoined by non-homologous end-joining, which may introduce a number of mutagenic base changes and (or) deletions. In addition to inform

ational loss at the base level, the DSB destroys the structural continuity necessary for faithful segregation of genetic information to daughter cells; loss of a centromere or teleomere or rejoining unrelated DNA ends may result in

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daughter cells with defective genomes (Obe et al. 2002). The C-terminal end of histone H2AX is intimately involved in the efficient recognition and repair of chromatin regions containing DSBs (Redon et al. 2002). Celeste et al. (2002) demonstrated that although mice lacking H2AX are viable, they are defective in several important processes. $H2AX^{\Delta/\Delta}$ mice are smaller and sensitive to ionizing radiation. They are defective in class switch recombination but competent in V(D)J recombination. $H2AX^{\Delta/\Delta}$ females are fertile, but $H2AX^{\Delta/\Delta}$ males are sterile. During meiosis, sperm production requires the endonuclease Spo11 to place DSBs in chromosomes so that homologous chromosomes may synapse. In $Spo11^{\Delta/\Delta}$ males γ-H2AX is absent over the autosomes but still present over the sex body, suggesting H2AX plays a unique role in sex body formation (Mahadevaian et al. 2001). H2AX is necessary for G2/M checkpoint competency at physiological doses of ionizing radiation (Fernandez-Capetillo et al. 2002). $H2AX^{\Delta/\Delta}$ cells display substantially increased numbers of genomic defects in mitotic spreads (Celeste et al. 2002; Bassing et al. 2002). These characteristics demonstrate the importance of understanding the parameters and functions of γ-H2AX formation at sites of DSBs. This article discusses DSB formation by various processes and the parameters of γ-H2AX foci formation.

DNA DSBs from diverse causes have diverse structures

DNA DSBs are not a homogeneous class of lesions but may differ in the structure of the resulting broken ends depending on the origin of the break. The DSBs most commonly referred to as DNA damage are usually accidental, caused by exogenous or endogenous agents, often high energy particles and rays from radioactive disintegration. Cosmic radiation is responsible for a DNA DSB in about 10^5 cells in a typical human each second. Since a typical human contains about 10^{14} cells, 10^5 cells are a small fraction, but in about 20 years, each of the 10^{14} cells would on average receive one DSB. Radiation sources can also be endogenous. Potassium is an essential component of living tissue, yet one isotope ^{40}K is radioactive with a half-life of 1.26×10^9 years. Thus about 8% of the amount of ^{40}K generated in the big bang is still around, and enough of it is in us to cause a DSB in 2×10^4 cells each second. Some of these DSBs may result in cell alterations leading ultimately to cancer. In 1955, Isaac Asimov (1955) published a speculation that life only became possible as ^{40}K decayed.

A great deal of study has gone into the structures of radiation-induced DNA lesions (Ward 1988). A highly energetic ionizing particle or ray penetrates a cell leaving a trail of collisions with cellular molecules, including water. Such collisions generate clusters of reactive oxygen species, and if a cluster forms within a few ångströms of a DNA double helix, free radicals may diffuse to the chromatin and generate random DNA damage. DSBs are the least common of the types of damage but are considered to be the most serious and potentially lethal. In addition, compared with metabolic DSBs, radiation-induced DSBs are more heterogeneous — some being easy and others impossible to repair. This heterogeneity results from the random collateral DNA damage generated near the DSB site by other free radicals from the same cluster of reactive oxygen species. The existence of these sites, termed locally multiply damaged sites (Ward 1994), has been substantiated using enzymes specific for different types of DNA damage (Sutherland et al. 2000*a*; Sutherland et al. 2000*b*). Of the DNA lesions in locally multiply damaged sites containing a DSB, 80% were composed of single strand breaks, altered bases, and damaged deoxyribose backbones. This heterogeneity may be the source of the biphasic kinetics of DSB rejoining as measured by pulsefield gel electrophoresis; the halftime of the rapid phase is about 10 min, while that of the slow phase is about 2 h.

However, living systems also introduce DSBs under controlled circumstances for essential processes. Homologous recombination during meiosis is fundamental in rearranging the genetic material for future generations. The processes of V(D)J and class switch recombination also purposely introduce DSBs into the DNA to generate novelty in the immune system. In addition to the highly programmed processes, some DSBs may be introduced as accidents of metabolism, such as those formed when topoisomerase I-DNA complexes collide with replication forks. Perhaps because of the diverse origins and chemistry of DSBs, cells may have evolved a general mechanism for recognizing any type of DSB without regard to the chemistry at the break. As far as has been examined, γ-H2AX forms at sites of all types of DSBs.

DNA DSB recognition and repair takes place in chromatin

In eucaryotes, the physical macromolecular complex containing nuclear DNA is chromatin whose fundamental unit in both a structural and functional sense is the nucleosome. The nucleosome is composed of 145 bp DNA and eight small basic protein molecules called histones, two from each of the four core histone families, H4, H3, H2B, and H2A. A minimum of another 20 bp of DNA complexed with the linker histone H1 stretches between nucleosomes. The result is that the 4 m of DNA in a mammalian G2 cell is packaged into 180 mm of 30 nm diameter fibers and is further condensed to 120 μm of 700 nm diameter arms of mitotic chromosomes.

More recently, the nucleosome has also come to be appreciated for its roles in the control of information flow from the DNA (Jenuwein and Allis 2001). These include not only the regulation of transcription, but also the regulation of the condensation state for meiosis and mitosis, and the maintenance of genome integrity. These roles are mediated by modifications of specific amino acid residues, located primarily but not exclusively in the N-terminal tails, and by the presence of specialized histone species which confer particular properties on the chromatin. Thus not only are the nucleosomes essential in packaging the DNA, they are also essential in controlling when and where the package is opened (Felsenfeld and Groudine 2003). That histones contain modified amino acids has been known since they were first sequenced (Wu et al. 1986). Although researchers had recognized that the complex modification patterns and sequence variations of the histone proteins gave multitudinous opportunities for the regulation of chromosome functions, only recently have the tools and techniques become available

to relate these alterations to specific functions. Antibodies specific to site-specific modifications coupled with ChIP techniques have made it possible to study the effects of a particular histone modification on a particular gene region in chromatin, providing direct experimental support for the theory of the histone code (Jenuwein and Allis 2001).

H2AX, unique among members of the histone H2A family, contains a conserved C-terminal tail that is involved in localizing and repairing DNA DSBs (Redon et al. 2002) (Fig. 1). In yeast, two genes, HTA1 and HTA2, encode the H2AX ortholog while in mammals, one gene encodes the H2AX protein. The distribution of the H2AX orthologs is different between yeasts and mammals. In the former, the H2AX orthologs are 95% of the H2A complement but are only 2–10% in the latter, most of the remainder being the H2A1 and H2A2 species encoded by 11 genes in the human (Fig. 1; human 1(L) and (P)). There are also other H2A variants whose functions are still unclear. One is H2AZ which is 5–10% of the H2A complement in both yeast and mammals. In addition, mammals contain several other minor H2A species not reported in yeast. Two, macroH2A1 (Pehrson and Fried 1992) and macroH2A2 (Costanzi and Pehrson 2001) were identified in the 1990s and one, H2A-Bbd (Chadwick and Willard 2001), just recently. Whether there are more remains to be seen.

Generally the conserved core regions of the histone proteins are involved in histone–histone interaction, while the N-terminal and sometimes the C-terminal tails interact with DNA. There are three amino acid residues in the core and N-terminal tail that are unique to mammalian H2AX; a threonine residue at position 6, a histidine residue at position 38, and a glycine residue at position 99 (Fig. 1, underlined residues in the human H2AX sequence). Of these the histidine residue is in a critical region for H2A–H2A interaction in the nucleosome as found for the H2AZ species; the sequence differences between H2AZ and other H2A species in this region possibly require two H2AZ molecules to be present in the same nucleosome (Suto et al. 2000). Whether that is the case for H2AX is not known.

The evolution of the histone H2AX C-terminal tail is shown in Fig. 1. The tail has two regions, a highly conserved C-terminal tip of four amino acids containing an invariant serine four residues from the C-terminus and a linker of varying length and sequence that links the conserved C-terminal tip with the conserved core. The linker length varies between 19 in mammals and 3 in *Giardia intestinalis*. Mammals also contain a second site three residues away from the main site; the impact of this second site is not known (Rogakou et al. 1998). When a DSB occurs, many molecules of histone H2AX in the chromatin adjacent to the break become phosphorylated on the invariant serine. H2AX phosphorylated on this serine, residue 139 in mammals, is named γ-H2AX. In the yeast H2AX orthologs HTA1 and HTA2 contain the conserved serine at residue 129 and are named γ-H2A when phosphorylated.

Parameters of γ-H2AX formation

Because of the demonstrated importance of γ-H2AX in efficient DSB recognition and repair, it is of general interest to know the specific parameters of its formation. The evidence indicates that γ-H2AX forms whenever a DSB is formed, regardless of the origin of the break. Two techniques are important in measuring γ-H2AX formation. First, γ-H2AX can be resolved from other forms of H2AX by two-dimensional histone gel electrophoresis, making it possible to calculate a stoichiometry of γ-H2AX formation. Second, with an antibody raised to the phosphorylated C-terminal peptide of H2AX, numbers of γ-H2AX nuclear foci can be measured.

When is γ-H2AX formed?

Two-dimensional gel analysis of histones extracted from mammalian cells at various times demonstrated that γ-H2AX is detectible within 3 min after irradiation. The fraction of H2AX that becomes phosphorylated rapidly increases until a plateau is reached 10–30 min after irradiation, the level of the plateau being proportional to dose (Rogakou et al. 1998). An antibody reveals that γ-H2AX molecules are found as nuclear foci with the number of foci per cell proportional to the number of introduced DSBs. Foci of γ-H2AX also appeared within 3 min after irradiation (Rogakou et al. 1999).

Where is γ-H2AX located?

Although the result that the numbers of γ-H2AX foci are proportional to the numbers of introduced DSBs suggests that the foci might be coincident with the break sites, the proof of this came from a novel technique to direct DSBs to particular nuclear regions. DSBs can be introduced into cells grown in the presence of BrdU when these cells are incubated with Hoechst dye 33258 and then exposed to UVA light of wavelength 365 nm (Limoli and Ward 1993). If the light is delivered by a 0.5 μm diameter laser beam of appropriate wavelength through a microscope, the damage can be placed in distinct areas of the nucleus. γ-H2AX is found along the path of the beam, indicating that it forms adjacent to break sites (Rogakou et al. 1999).

How many foci per DSB?

Since ionizing radiation generates a heterogeneous mixture of DNA lesions, the number of γ-H2AX foci per cell was calibrated to the number of DSBs using ^{125}IUdr incorporated into the DNA. Because ^{125}I decays by the Auger mechanism in which an inner shell electron is captured by the nucleus, a localized but intense disruption takes place that results in a DSB with over 90% efficiency. Thus ^{125}I decays can be taken as a direct measure of introduced DSBs. This approach verified that each γ-H2AX focus corresponds to one DSB (Sedelnikova et al. 2002).

How many H2AX molecules are involved per focus?

The fraction of H2AX phosphorylated was found to be proportional to the number of DSBs introduced per cell with about 0.03% of the H2AX becoming phosphorylated per DSB introduced, a value indicating that hundreds to thousands of H2AX molecules become phosphorylated per DSB. A normal human fibroblast with H2AX as 10% of its H2A complement contains about 6×10^6 molecules of H2AX per cell. Thus about 2000 H2AX molecules are phosphorylated per DSB, indicating that the signal announcing the presence of a DSB is highly amplified (Rogakou et al. 1998).

Fig. 1. Sequence comparison of histone H2AX from different species. Sequences were taken from GenBank and manually aligned. Residue numbers are shown across the top. H2AX regions are delineated below the sequences. H2AX is composed of a conserved core and a C-terminal tail, which in turn is composed of a linker, evolutionarily variable in both length and sequence, and a conserved tip. Underlining in the human H2AX sequence N terminal to residue 119 denotes the three amino acid differences between it and the human H2A1 species.

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Residue number              10        20        30        40        50        60        70
HUMAN  1(P)          SGRGKQGGKARAKAKTRSSRAGLQFPVGRVHRLLRKGNYAERVGAGAPVYLAAVLEYLTAEILELAGNAARDNKKTRII
HUMAN  1(L)          SGRGKQGGKARAKAKSRSSRAGLQFPVGRVHRLLRKGNYSERVGAGAPVYLAAVLEYLTAEILELAGNAARDNKKTRII
HUMAN  X             SGRGKTGGKARAKAKSRSSRAGLQFPVGRVHRLLRKGHYAERVGAGAPVYLAAVLEYLTAEILELAGNAARDNKKTRII

Residue number       80        90        100       110       120       130       140
HUMAN  1(P)          PRHLQLAIRNDEELNKLLGRVTIAQGGVLPNIQAVLLPKKTESHHKAKGK*
HUMAN  1(L)          PRHLQLAIRNDEELNKLLGKVTIAQGGVLPNIQAVLLPKKTESHHKAKGK*
HUMAN  X             PRHLQLAIRNDEELNKLLGGVTIAQGGVLPNIQAVLLPKKTSATVGPKAPSGGKKATQASQEY*
MOUSE X              PRHLQLAIRNDEELNKLLGGVTIAQGGVLPNIQAVLLPKKSSATVGPKAPAVGKKASQASQEY*
SPRUCE               PRHIQLAVRNDEELSKLLGAVTIANGGVLPNIHQVLLPKK''''''''SGKDKGEIGSASQEF*
CRESS X              PRHIQLAVRNDEELSKLLGSVTIANGGVLPNIHQTLLPSK''''''''VGKNKGDIGSASQEF*
CHICKPEA             PRHIQLAVRNDEELSKLLGSVTIANGGVLPNIHQTLLPKK''''''''VGKGKGIGSASQEF*
TETRA PY1            PRHILLAIRNDEELNKLMANTTIADGGVLPNINPMLLPSK''''''''SKKTESRGQASQDI*
TETRA TH1            PRHILLAIRNDEELNKLMANTTIADGGVLPNINPMLLPSK''''''''SKKTESRGQASQDL*
FUNGI(B.F)           PRHLQLAIRNDEELNKLLGHVTIAQGGVLPNIHQSLLPKK''''''''TAKTAGGKPASQEL*
ASPERGILLUS          PRHLQLAIRNDEELNKLLGHVTIAQGGVLPNIHQNLLPKK''''''''''TPKAGKGSQEL*
BUDDINGYEAST 1       PRHLQLAIRNDDELNKLLGNVTIAQGGVLPNIHQNLLPKK''''''''''SAKATKASQEL*
BUDDINGYEAST 2       PRHLQLAIRNDDELNKLLGNVTIAQGGVLPNIHQNLLPKK''''''''''SAKTAKASQEL*
FISSIONYEAST 1       PRHLQLAIRNDEELNKLLGHVTIAQGGVVPNINAHLLPKT''''''''''SGGTGKPSQEL*
FISSIONYEAST 2       PRHLQLAIRNDEELNKLLGHVTIAQGGVVPNINAHLLPKQ''''''''''SGKGKPSQEL*
GIARDIA INTEST       PNHILTALRKDKELATIFANVTIREGGVARSAKEGREGKG                SHRSQDL*

H2AX regions                          Conserved core     >|<   tail              >|
                                                          |<   linker        >|tip|
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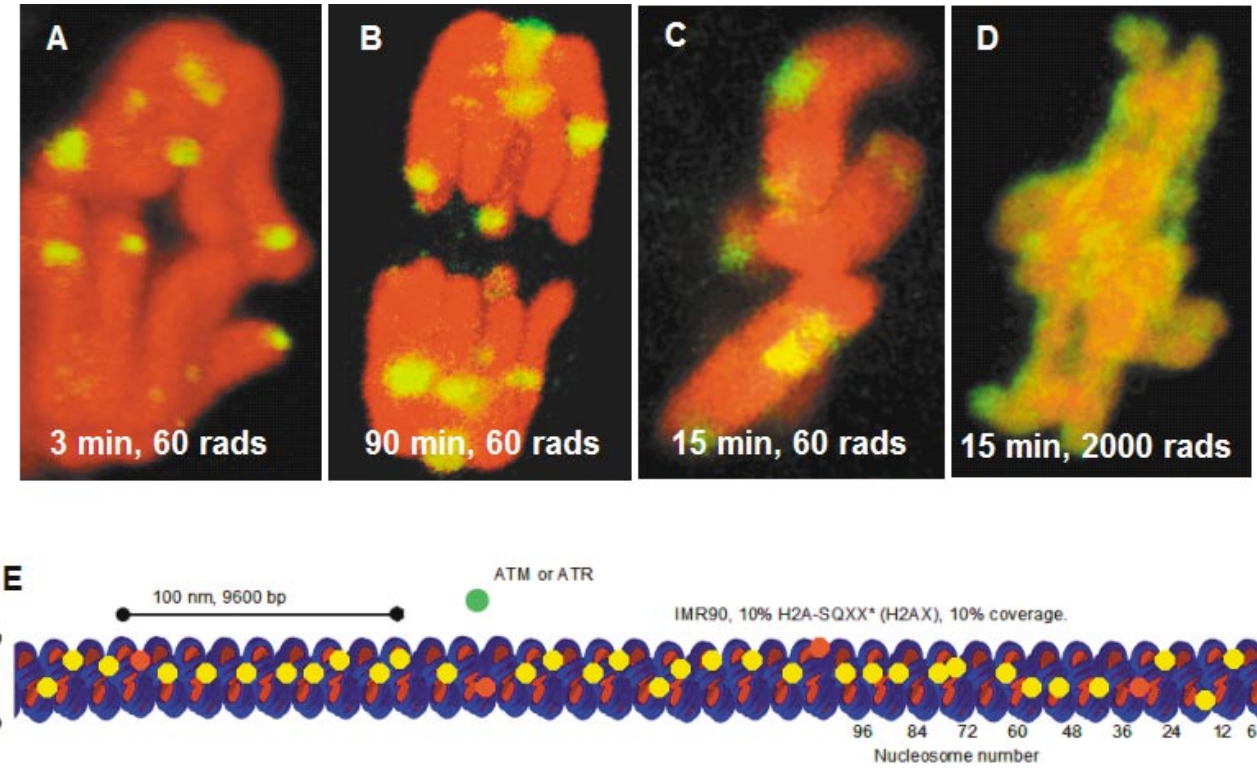
How much chromatin is involved per focus?

Assuming that H2AX is randomly distributed in chromatin and that every contiguous H2AX molecule near the DSB is phosphorylated, the value of 2000 γ-H2AX molecules corresponds to chromatin containing approximately 2 Mb of DNA. To check this prediction, we were able to use a cell line derived from the Indian muntjac that contains a diploid chromosome number of 6. Interestingly, the Indian muntjac can make viable, although sterile, offspring with the Chinese muntjac, which has 46 chromosomes, indicating that the Indian muntjac has fused many chromosomes together to form a few with up to 10^9 bp per arm. Because each of the 6 chromosome arms can be distinguished in mitotic cells, muntjac cell cultures are a particularly advantageous system in which to investigate some characteristics of γ-H2AX foci. Thus while stoichiometry studies indicate that an amount of H2AX became phosphorylated equivalent to about 2 Mb of chromatin, measurements of foci size in mitotic chromosomes indicate considerably larger regions are involved (Figs. 2A–2D). The foci in Fig. 2B are at least 0.5 μm long and as such would comprise about 30 Mb of chromatin. These findings can be reconciled if it is assumed that even in a focus not every H2AX molecule becomes phosphorylated. Figure 2E shows a model that brings together the known information about γ-H2AX foci formation. The two relevant points as applied to γ-H2AX distribution in irradiated mammalian cells are that one in five nucleosomes contains an H2AX molecule (assuming one H2AX molecule per nucleosome) but only one in 10 of those H2AX molecules are phosphorylated, resulting in a ratio of about one γ-H2AX molecule for every 50 nucleosomes in a focus. These results indicate that the signal announcing the presence of a DSB is even more highly amplified, first by the number of γ-H2AX molecules and second by their distribution along a large amount of chromatin in a focus (Rogakou et al. 1999).

Does a DSB necessarily mean chromosome fragmentation?

Figures 2A and 2B show foci in muntjac mitotic chromosomes either 90 or 3 min after irradiation with 60 rad (1 rad = 10 mGy). Several characteristics are apparent. First, 60 rad is expected to introduce about 20 DSBs of which about 12 are not repaired in the first few minutes. Second, although this mitotic figure contains a number of foci consistent with the number of DSBs expected for 60 rad, the arms appear continuous — not broken or in any visible way discontinuous at the position of these foci. When a chromosome arm is broken off, there is a large focus at one end of the fragment (Rogakou et al. 1999), but in the vast majority of cases there is no apparent difference in DNA staining that might denote a break site. This suggests that a DSB in the DNA does not necessarily involve a separation of chromosomal fragments. That the lack of visible discontinuities may be a result of γ-H2AX focus formation is indicated by the finding that $H2AX^{\Delta/\Delta}$ mouse embryo fibroblasts contain a greater number of chromosomal abnormalities that the wild type cells. There are two ways that γ-H2AX foci could help decrease the incidence of chromosomal abnormalities. One is to keep the broken ends in position, making repair more likely. Another is to recruit a greater number of repair factors, speeding up DSB repair. Both processes could occur simultaneously. Such processes may also be used to advantage where DNA ends several kilobases apart are bought together such as in V(D)J and class switch recombination.

Fig. 2. Focus structure. (A–D) Images of muntjac mitotic chromosomes were recorded as described in Rogakou et al. (1998). Cultures of muntjac cells were fixed at the noted times after receiving the noted amount of ionizing radiation. The fixed cultures were scanned by eye for mitotic cells and the images recorded. (E) A model of the 30 nm fiber and the distribution of H2AX (yellow circles) and γ-H2AX (red circles) molecules in a focus.



How do foci grow?

H2AX is phosphorylated by the PI-3 kinase family members, ATM, ATR, and DNA-PK (Redon et al. 2002). Foci are initially visible (Fig. 2A, 3 min) as small compact structures, presumably close to the site of the break and grow along the chromatin strand away from the break (Fig. 2B, 90 min), finally stabilizing as thick discs traversing the chromosome arm. Several models can be presented to account for the apparent limit to focus size. First, there could be a discontinuity in the chromatin fiber structure, which forms a physical barrier to phosphorylation. A second possibility is that kinases track along the fiber but at some point fall off, forming an apparent kinetic boundary. A third possibility is that kinases coat the chromatin fiber, and a signal is transmitted, perhaps by a change in chromatin structure, to kinases further from the break; finally the signal decays. At present there is no information to favor one or another of these models. A candidate phosphatase has been characterized (Siino et al. 2002).

Is there a limit to foci formation?

Foci numbers are proportional to the amount of irradiation at doses likely to be relevant to mammals, but there is a point at which individual foci are no longer visible (Figs. 2C and 2D). In cells receiving 60 rad, a metaphase cell shows discrete foci on the chromosome arms on the metaphase plate, usually on just one of the two sister chromatids. However, a similar cell exposed to 2000 rad does not contain individual foci but a diffuse covering of γ-H2AX. Clearly the process fails at some point; however, it is useful to remember that 2000 rad is a superlethal dose of radiation for mammals — not relevant to their survival.

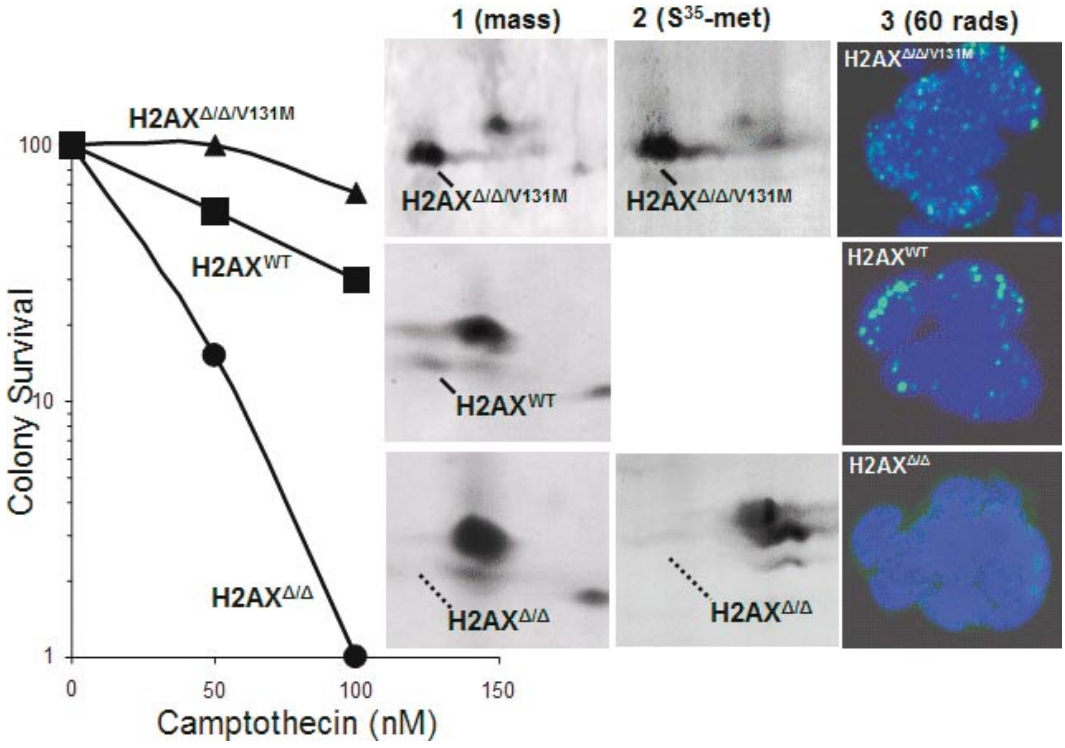
Which residues of H2AX are necessary for foci formation?

Like mouse embryo fibroblasts, $H2AX^{\Delta/\Delta}$ ES cells also demonstrate increased chromosome instability and sensitivity to ionizing radiation (Bassing et al. 2002). $H2AX^{\Delta/\Delta}$ ES cells are also hypersensitive to camptothecin (Fig. 3A) and fail to form foci after irradiation (Fig. 3B). To analyze restored $H2AX^{\Delta/\Delta}$ cells containing an H2AX transgene, a marked H2AX gene was designed in which residue 131 is altered to methionine, a residue absent from native H2AX. Thus by labeling a growing culture with [^{35}S]methionine, the source of protein produced can be verified to be a product of the transgene. $H2AX^{\Delta/\Delta V131M}$ ES cells expressing the H2AX-V131M transgene are resistant to camptothecin and form foci after irradiation as do $H2AX^{WT}$ ES cells (Fig. 3). In this manner, a marked H2AX transgene can serve as a basis for investigating the relative importance of tail length and of the various residues surrounding serine residues 136 and 139.

Speculation

Several general models will be mentioned concerning possible roles for γ-H2AX. First, the foci may help keep broken DNA ends together. γ-H2AX foci increase in size over about 30 min during which time a substantial number of DSBs

Fig. 3. Restoration of $H2AX^{\Delta/\Delta}$ ES cell wild type characteristics with a marked transgene. $H2AX^{WT/\Delta}$ ES cells cultures were obtained with a knockout vector as described in Celeste et al. (2002). The vector contained a gene for neomycin resistance. $H2AX^{\Delta/\Delta}$ ES cells were obtained with a second vector containing a gene for hygromycin resistance. An H2AX transgene with a V131M substitution to insert a unique methionine residue into the protein and with 988 bp of sequence upstream of the ATG site was transfected into $H2AX^{\Delta/\Delta}$ ES cells with a puromycin selection vector to form $H2AX^{\Delta/\Delta/transV131M}$. Cultures were selected, labeled overnight with [^{35}S]methionine and assayed by 2D gel electrophoresis for the presence of a novel S^{35}-containing spot in the area of the gel near the location of H2AX. Camptothecin resistance of the cultures was measured by colony survival after 7 days growth in the noted concentrations of camptothecin. Foci formation was measured in cultures fixed 30 min after being exposed to 60 rad. $H2AX^{WT}$ (squares), wild type ES cells; $H2AX^{\Delta/\Delta}$ (circles), ES cells with both genomic H2AX genes disrupted; $H2AX^{\Delta/\Delta/trans\text{-}V131M}$ (triangles), $H2AX^{\Delta/\Delta}$ cells transfected with the $H2AX^{V131M}$ transgene. The images in column 1 are Coomassie blue mass stain, in column 2 autoradiographs, in column 3 confocal images of ES cells stained for DNA (blue) and for γ-H2AX (green).



have been rejoined, but a number have not. By helping to keep broken DNA ends together, γ-H2AX may make successful and faithful repair more likely. If DNA ends drift apart, inappropriate chromatin fragments may be joined, resulting in translocations and other abnormalities. A related role for γ-H2AX foci may be to differentiate broken DNA ends from telomeres. Some abnormalities seen in metaphase spreads could be explained by telomere formation at a broken DNA ends.

It has been generally established that γ-H2AX foci attract repair factors (Paull et al. 2000), assembling a higher concentration of repair proteins near a DSB site. The more persistent the breaks, the larger the γ-H2AX foci and presumably the higher concentration of repair proteins. In addition, foci might be catalysts for macromolecular complex formation from smaller factors that diffuse more quickly through the nucleoplasm. For example, a 50 kDa protein has a diffusion constant of about 15 $\mu m^2/s$ in nucleoplasm, and thus might be expected to traverse the diameter of a nucleus on a time scale of seconds. Since larger complexes diffuse substantially more slowly and could also be selectively hindered by a dense network of chromatin fibers, larger complexes might accumulate more slowly at foci. Thus there could be an advantage for more rapidly diffusable small factors to accumulate at a focus and then assemble into large complexes. In this manner, γ-H2AX foci might amplify a signal by being a catalyst for complex formation.

References

Asimov, I. 1955. The radioactivity of the human body. Journal of Chemical Education, February 1955.

Bassing, C.H., Chua, K.F., Sekiguchi, J., Suh, H., Whitlow, S.R., Fleming, J.C., Monroe, B.C., Ciccone, D.N., Yan, C., Vlasakova, K., Livingston, D.M., Ferguson, D.O., Scully, R., and Alt, F.W. 2002. Increased ionizing radiation sensitivity and genomic instability in the absence of histone H2AX. Proc. Natl. Acad. Sci. U.S.A. **99**: 8173–8178.

Celeste, A., Petersen, S., Romanienko, P.J., Fernandez-Capetillo, O., Chen, H.T., Reina-San-Martin, B., Meffre, E., Difilippantonio, M.J., Sedelnikova, O.A., Redon, C., Pilch, D.R., Olaru, A., Tessarollo, L., Eckhaus, M., Camerini-Otero, R.D., Livak, F., Manova, K., Bonner, W.M., Nussenzweig, M., and Nussenzweig, A. 2002. Genomic instability in mice lacking histone H2AX. Science (Washington, D.C), **296**: 922–927.

Chadwick, B.P., and Willard, H.F. 2001. A novel chromatin protein, distantly related to histone H2A, is largely excluded from the inactive X chromosome. J. Cell Biol. **152**: 375–384.

Costanzi, C., and Pehrson, J.R. 2001. MACROH2A2, a new member of the MARCOH2A core histone family. J. Biol. Chem. **276**: 21 776 – 21 784.

Felsenfeld, G., and Groudine, M. 2003. Controlling the double helix. Nature (London), **421**: 448–453.

Fernandez-Capetillo, O., Chen, H.T., Celeste, A., Ward, I., Romanienko, P.J., Morales, J.C., Naka, K., Xia, Z., Camerini-Otero, R.D., Motoyama, N., Carpenter, P.B., Bonner, W.M., Chen, J., and Nussenzweig, A. 2002. DNA damage-induced G2-M checkpoint activation by histone H2AX and 53BP1. Nat. Cell Biol. **4**: 993–997.

Jackson, S.P. 2002. Sensing and repairing DNA double-strand breaks. Carcinogenesis, **23**: 687–696.

Jenuwein, T., and Allis, C.D. 2001. Translating the histone code. Science (Washington, D.C), **293**: 1074–1080.

Limoli, C.L., and Ward, J.F. 1993. A new method for introducing double-strand breaks into cellular DNA. Radiat. Res. **134**: 160–169.

Mahadevaian, S.K., Turner, J.M.A., Rogakou, E.P., Baudat, F., Blanco-Rodríguez, J., Jasin, M., Bonner, W.M., and Burgoyne, P.S. 2001. Initiation of homologous recombination precedes synapis during mammalian meiosis. Nat. Genet. **27**: 271–276.

Obe, G., Pfeiffer, P., Savage, J.R., Johannes, C., Goedecke, W., Jeppesen, P., Natarajan, A.T., Martinez-Lopez, W., Folle, G.A., and Drets, M.E. 2002. Chromosomal aberrations: formation, identification and distribution. Mutat. Res. **25**: 17–36.

Paull, T.T., Rogakou, E.P., Yamazaki, V., Kirchgessner, C.U., Gellert, M., and Bonner, W.M. 2000. A critical role for histone H2AX in sequential recruitment of repair factors to nuclear foci after DNA damage. Curr. Biol. **10**: 886–895.

Pehrson, J.R., and Fried, V.A. 1992. MacroH2A, a core histone containing a large nonhistone region. Science (Washington, D.C), **257**: 1398–1400.

Redon, C., Pilch, D., Rogakou, E., Sedelnikova, O., Newrock, K., and Bonner, W. 2002. Histone H2A variants H2AX and H2AZ. Curr. Opin. Genet. Dev. **12**: 162–169.

Rogakou, E.P., Pilch, D.R., Orr, A.H., Ivanova, V.S., and Bonner, W.M. 1998. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J. Biol. Chem. **273**: 5858–5868.

Rogakou, E.P., Boon, C., Redon, C., and Bonner, W.M. 1999. Megabase Chromatin Domains Involved in DNA Double-strand Breaks in vivo. J. Cell Biol. **146**: 905–916.

Rothkamm, K., and Lobrich, M. 2002. Misrepair of radiation-induced DNA double-strand breaks and its relevance for tumorigenesis and cancer treatment. Int. J. Oncol. **21**: 433–440.

Sedelnikova, O.A., Rogakou, E.P., Panuytin, I.G., and Bonner, W.M. 2002. Quantitative detection of ^{125}IUdr-induced DNA double-strand breaks with γ-H2AX antibody. Radiat. Res. **158**: 486–492.

Siino, J.S., Nazarov, I.B., Svetlova, M.P., Solovjeva, L.V., Adamson, R.H., Zalenskaya, I.A., Yau, P.M., Bradbury, E.M., and Tomilin, N.V. 2002. Photobleaching of GFP-labeled H2AX in chromatin: H2AX has low diffusional mobility in the nucleus. Biochem. Biophys. Res. Commun. **297**: 1318–1323.

Sutherland, B.M., Bennett, P.V., Sidorkina, O., and Laval, J. 2000*a*. Clustered damages and total lesions induced in DNA by ionizing radiation: oxidized bases and strand breaks. Biochemistry, **39**: 8026–8031.

Sutherland, B.M., Bennett, P.V., Sidorkina, O., and Laval, J. 2000*b*. Clustered DNA damages induced in isolated DNA and in human cells by low doses of ionizing radiation. Proc. Natl. Acad. Sci. U.S.A. **97**: 103–108.

Suto, R.K., Clarkson, M.J., Tremethick, D.J., and Luger, K. 2000. Crystal structure of a nucleosome core particle containing the variant histone H2A.Z. Nat. Struct. Biol. **7**: 1121–1124.

Ward, J.F. 1988. DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and reparability. Prog. Nucleic Acid Res. Mol. Biol. **35**: 95–125.

Ward, J.F. 1994. The complexity of DNA damage: relevance to biological consequences. Int. J. Radiat. Biol. **66**: 427–432.

Wu, R.S., Panusz, H., Hatch, C.L., and Bonner, W.M. 1986. Histones and their modifications. *In* Critical reviews in biochemistry and molecular biology. Vol. 20. *Edited by* G.D. Fasman. CRC Press, Boca Raton, Fla. pp. 201–263.