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Spots, Damn'd spots and γH2AX foci

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Comment on: Martín M, et al. γH2AX foci on apparently intact mitotic chromosomes: Not signatures of misrejoining events but signals of unresolved DNA damage. Cell Cycle 2014; 13(19):3026–36; http://dx.doi.org/10.4161/15384101.2014.947786

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". . ..Out, damn'd spot! out, I say!—One; 2: why. . ." [1]

As Shakespeare has the Queen of Scotland so prophetically proclaim, the physiological nature of residual spots (foci) continues to frustrate DNA researchers long after the 17th century.

The immunohistochemical labeling of proteins involved in the repair of DNA double strand breaks (DSBs) reveals a very reproducible dose- and time dependent accumulation and disappearance of microscopic protein aggregates at discrete sites (foci) in the chromatin. However, we ignore at our peril the fact that such a convenient and reproducible marker of DSB damage remains a surrogate that neither detects the double-stranded break itself, nor informs on the physiological purpose. Thus, DSBs arising from lagging strand replication, collapsed replication forks, meiotic recombination, gene conversion and rearrangements are not distinguishable from radiation-induced DSBs.

The presence of foci after repair is concluded presents an additional conundrum. As these persistent foci are typically much larger than foci at sites of active repair they have been suggested to mark unrepaired or misrepaired DNA.[2] Their co-localization with heterochromatin suggests they may also be playing a role in DNA packing.[3] These 2 non-exclusive possibilities have consequence for understanding both the function of DNA repair proteins and the role of mis- or un-repaired DNA in carcinogenesis. In the latter case, it is suggested that ionizing radiation can, under certain circumstances, generate complex DNA lesions that cannot be correctly handled by DNA repair.[4] The presence of such unrepaired DNA has been linked to entry into a form of cellular senescence.[5] If such damaged cells nevertheless do progress through mitosis, non-rejoined DNA fragments will very likely be lost during chromosomal segregation in telophase. For cells in highly proliferative tissues or organs (haematopoietic system, small intestinal epithelium, skin, mammary gland), this can have two major consequences. Either such cells lose their division potential (causing anaplasia or degeneration), or cells may form aberrant clones, with the risk for malignant transformation.

The paper by Martín et al[6] now sheds some more light onto the nature of these persistent foci. Using a combination of FISH and immunolabelling in post-irradiated cycling cells they have established that interstitial foci retained after DNA synthesis in mitotic cells do not mark sites of macroscopic chromosomal damage, nor do they mark sites of incorrect chromosome rejoining during an already completed DSB repair. Intriguingly however, analysis of the repair proteins present at these mitotic foci shows that γH2AX and MRE11 are both retained, while 53BP1 is absent. This is assumed to indicate ejection of 53BP1 from an unrepaired DSB at the G2/M transition, but may equally indicate failure to correctly initiate formation of the repair complex at an earlier stage. In either case this provides further supportive evidence that persistent DSB foci include some sites where damage remains unrepaired, possibly due to complexity of the damage. Using image analysis to estimate the size of repair foci Martín and colleagues also report large variation, which appear to correlate to some extent with the chromosomal location. The visible chromatid breaks in metaphase chromosomes were mostly decorated by large foci, presumably due to the persistent labeling of large tracts of DNA by γH2AX. However, some of these chromatid sites were marked by medium sized foci, while smaller foci were found much less frequently. The apparently inverse situation was reported at sites of persistent foci that did not correlate with chromatid damage. These sites revealed an inverse distribution of foci sizes, with more smaller than medium foci and only infrequent large foci. It is tempting to speculate that the scale of the focus represents the size of the unrepaired lesion at the point at which repair was terminated.

The paper of Martín et al answers the question of persistent foci marking misrepaired DNA joins with a "no", but raises several new questions, including the role of persistent foci in the induction of senescence. It has recently been suggest that PML bodies may accumulate at sites of unresolved repair foci,[7] probably via recruitment by MRE11, but paradoxically these do not appear to influence senescence. Could the arrival of PML be linked to the apparent ejection of 53BP1? Are the retained sites still attempting to repair, as has been shown by the presence of phosphorylated ATM at persistent foci in the skin?[8]

References

1. Shakespeare W. Macbeth. Ed. Thomas Marc Parrott. American Book Co.: New York; 1904; p. 147.
2. Suzuki M, et al. Biochem Biophys Res Commun 2006; 340:145–50; PMID:16360120; http://dx.doi.org/10.1016/j.bbrc.2005.11.167
3. Schuler N, et al. PLoS One 2013; 8(5):e63932; PMID:23691119; http://dx.doi.org/10.1371/journal.pone.0063932
4. Goodhead DT. Int J Radiat Biol 65:7–17; PMID:7905912; http://dx.doi.org/10.1080/09553009414550021
5. Sedelnikova OA, et al. Nat Cell Biol 2004; 6:168–170; PMID:14755273; http://dx.doi.org/10.1038/ncb1095
6. Martín M, et al. Cell Cycle 2014; 13(19); PMID:25486563; http://dx.doi.org/10.1371/10.4161/15384101.2014.947786
7. Münch S, et al. Molecular Cellular Biology 2014; 34:1733–1746; PMID:24615016; http://dx.doi.org/10.1128/MCB.01345-13
8. Ahmed EA, et al. PLoS One. 2012;7:e39521; PMID:22761813; http://dx.doi.org/10.1371/journal.pone.0039521