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Global chromatin fibre compaction in response to DNA damage

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ABSTRACT

DNA is protected by packaging it into higher order chromatin fibres, but this can impede nuclear processes like DNA repair. Despite considerable research into the factors required for signalling and repairing DNA damage, it is unclear if there are concomitant changes in global chromatin fibre structure. In human cells DNA double strand break (DSB) formation triggers a signalling cascade resulting in H2AX phosphorylation (γH2AX), the rapid recruitment of chromatin associated proteins and the subsequent repair of damaged sites. KAP1 is a transcriptional corepressor and in HCT116 cells we found that after DSB formation by chemicals or ionising radiation there was a wave of, predominantly ATM dependent, KAP1 phosphorylation. Both KAP1 and phosphorylated KAP1 were readily extracted from cells indicating they do not have a structural role and γH2AX was extracted in soluble chromatin indicating that sites of damage are not attached to an underlying structural matrix. After DSB formation we did not find a concomitant change in the sensitivity of chromatin fibres to micrococcal nuclease digestion. Therefore to directly investigate higher order chromatin fibre structures we used a biophysical sedimentation technique based on sucrose gradient centrifugation to compare the conformation of chromatin fibres isolated from cells before and after DNA DSB formation. After damage we found global chromatin fibre compaction, accompanied by rapid linker histone dephosphorylation, consistent with fibres being more regularly folded or fibre deformation being stabilized by linker histones. We suggest that following DSB formation, although there is localised chromatin unfolding to facilitate repair, the bulk genome becomes rapidly compacted protecting cells from further damage.

1. Introduction

In mammalian cells DNA is packaged with histone proteins into nucleosomes which fold to form a 30-nm diameter chromatin fibre that are further packaged into large scale chromatin structure [1]. Chromatin both protects the DNA from damage but also provides a regulated environment for nuclear processes such as transcription, replication and DNA repair. DNA double strand breaks (DSBs) lead to chromosomal fragmentation and cause genomic rearrangements if not repaired [2]. To maintain genome stability, cells possess a surveillance system called the DNA damage response (DDR) which recognises and repairs DNA damage and initiates check point events that control G1/S progression [3]. After the induction of DSBs either by ionising radiation or chemically by agents such as neocarzinostatin (NCS) the damage is sensed by a process that remains controversial but activates a signalling pathway via the PI3-kinase related protein kinases (PIKKs) ATM, ATR and DNA-PK. ATM becomes autophosphorylated triggering a signalling cascade promoting the phosphorylation of H2AX marking 1 Mb domains around the sites of damage, binding of the MDC1 adapter protein and recruitment of 53BP1.

In areas surrounding DSBs there is chromatin movement, cytologically visible localised expansion, and by LM/ESI (light microscopy/electron spectroscopic imaging), chromatin with the appearance of 10-nm fibres have been seen within repair foci [4]. There are also changes in chromatin associated proteins in response to DNA DSBs including the recruitment of HP1 and KAP1 to sites of damage in a p150CAF-1 dependent manner [5] that may function to reorganise chromatin. Furthermore loss of HP1 results in high sensitivity to DNA DSBs [6], possibly by making the chromatin more accessible to damage. KAP1 is an abundant nuclear protein that promotes the formation of transcriptionally repressed heterochromatin-like structures. In response to damage, KAP1 is phosphorylated in an ATM-dependent manner at damage sites, from where it spreads throughout the nucleus [7,8]. Heterochromatin provides a barrier for DNA repair and KAP1 phosphorylation is required for repairing damage in heterochromatin, but depletion of HP1 proteins alleviates the need for pKAP1 at heterochromatin [9,10] suggesting they are both involved in modulating chromatin structure.

Abbreviations: NCS, neocarzinostatin; DSB, double strand break; MNase, micrococcal nuclease.

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Changes in global chromatin organisation are seen in a number of physiological situations including the initiation of apoptosis, mitosis and the formation of facultative heterochromatin in the final stages of differentiation in nucleated erythrocytes. However, it is important to distinguish between changes in chromatin structure that occur at the level of the 30-nm chromatin fibre and higher levels of chromatin organisation. During facultative heterochromatin formation in chicken erythrocytes the variant linker histone H5 becomes dephosphorylated [11,12] promoting chromatin compaction by increasing electrostatic interactions [13] between histone proteins and the DNA. Ionising radiation also triggers the rapid but transient dephosphorylation of linker histones in Jurkat and RKO cells in an ATM dependent manner [14]. In contrast, during mitosis histone H1 becomes phosphorylated, possibly relaxing the chromatin fibre to enable remodelling to a subsequently more compact structure.

In response to DNA DSBs chromatin decondensation and a global increase in chromatin accessibility have been reported [15–17] and by LM/ESI DSBs cause a global decrease in chromatin density [4]. Furthermore, KAP1 depletion, or mimicking constitutive phosphorylation of this protein has been suggested to lead to constitutive global chromatin "relaxation" that might facilitate DNA damage repair [7]. However, global chromatin fibre relaxation would potentially render the DNA at greater risk of damage. To investigate whether DNA damage repair is accompanied by a global reconfiguration of fundamental chromatin fibres we have induced DNA damage and investigated the chromatin structure using nuclease sensitivity assays and a biophysical sedimentation approach that measures chromatin fibre compaction. Although we find a rapid change in KAP1 and H2AX phosphorylation we do not find a change in nuclease sensitivity of the chromatin fibres. However, we find that in response to DNA DSBs the chromatin fibre adopts a more compact structure, consistent with fibres being more regularly folded or fibre deformations being stabilized. This global compaction of chromatin fibres could therefore protect the genome from subsequent damage.

2. Materials and methods

2.1. Cell lines and reagents

Human HCT116 and U2OS cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum (FCS). All reagents were purchased from Sigma. The specific ATM inhibitor KU55933 and the specific DNA-PK inhibitor NU7441 were gifts from KUDOS. DNA damage was either introduced by treatment with the radiomimetic agent neocarzinostatin (NCS) (50–200 ng/ml) or by γ-irradiation (2–30 Gy) from a Co60 source. For colony forming assays cells were γ-irradiated or treated with NCS and plated at different densities. Cells were grown for 9 days, stained using 0.4% sulforhodamine B and the colonies counted.

2.2. Western blotting

Protein was fractionated on a 10% or 12% SDS polyacrylamide gel, transferred to Hybond P, and membranes were probed with antibodies that detect the following: KAP1 (1:1000; Bethyl Laboratories), pS824 KAP1 (1:1000; Bethyl Laboratories), H2AX (1:1000; 07-627, Millipore), γH2AX (1:500; 05-636, Millipore), HP1α (1:500; MAB3446, Millipore), HP1β (1:500; MAB3448; Millipore), hyperphosphorylated H1 (1:500; 06-597, Millipore), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1:2000, Abcam). Detection was performed by ECL.

2.3. Immunofluorescence

Human U2OS cells were grown on slides and fixed using 4% pFa in PBS [18]. The cells were permeabilized using Triton X-100 in PBS and were sequentially incubated with pS824 KAP (1:100), γH2AX (1:100) and secondary antibodies (Jackson Laboratories).

2.4. Preparation and fractionation of cells, nuclei and chromatin

To fractionate cells into supernatant and pellet fractions, cells were scrapped into gentle lysis buffer (PBS supplemented with 0.5% TX-100, 5 mM EDTA, 200 μM PMSF) and incubated for 30 min with mixing. The samples were centrifuged at max speed for 5 min. Supernatant and pellets were taken and resuspended in equivalent volumes 2× SDS sample buffer, boiled and sonicated. Nuclei were prepared as described [18]. For micrococcal nuclease (MNase) sensitivity digests, the nuclei concentration was adjusted to 4 A260 in nuclei buffer R. About 50 U/ml MNase (Worthington) was added, and aliquots were removed into stop buffer (2% SDS, 200 μg/ml proteinase K, and 10 mM EDTA) at various time intervals. Purified DNAs were fractionated on a 1% agarose gel in Tris–borate buffer in the presence of EtBr. Soluble chromatin was prepared and fractionated on 6–40% isokinetic sucrose gradients in TEEP80 buffer (10 mM Tris–HCl pH 8, 1 mM EDTA, 1 mM EGTA, 80 mM NaCl, 250 μM PMSF) as described previously [19,20]. Gel images were analysed using the Aida software package version 4.22 (Raytek).

3. Results and discussion

3.1. Chromatin modifications in response to DNA DSBs

Mammalian cells rapidly sense DNA damage and elicit a response. It has been suggested that part of this process is to trigger a global remodelling of chromatin structures to facilitate DNA damage repair. DNA strand breaks can be introduced by ionising radiation (e.g. γ-rays) or the radiomimetic agent neocarzinostatin (NCS). We have investigated the global effects of DNA DSBs in U2OS and HCT116 cells. Previously it has been shown that DNA damage promotes the rapid phosphorylation of KAP1 [7,8]. We confirmed this response in U2OS cells and in HCT116 cells. After treatment of cells with NCS there was a rapid phosphorylation of KAP1 which persisted for approximately 2 h before fading away (Fig. 1A). H2AX phosphorylation is one of the earliest events in the DNA damage response. After NCS treatment H2AX was rapidly phosphorylated and this mark persisted for up to 6 h. KAP1 phosphorylation was also induced by γ irradiation in HCT116 cells to an even greater extent, demonstrating these cells have a robust response to DNA DSBs (Fig. 1B). Furthermore, as KAP1 phosphorylation is stronger in HCT116 cells than U2OS cells it suggests there might be cell line specific differences. This could be due to an increased level of KAP1 in HCT116 cells or that these cells either have increased levels of DNA damage kinases or that these cells are particularly efficient at sensing DNA DSBs. To investigate whether the associated KAP1 with chromatin changes upon phosphorylation, cells were exposed to NCS and extracted in gentle lysis buffer. Soluble proteins are readily extracted whilst histones and tightly associated proteins remain in the pellet. KAP1 is phosphorylated after DNA damage but pKAP1 remains in the soluble fraction (Fig. 1C) suggesting that KAP1 has a role in signalling DNA damage response rather than becoming structurally associated with chromatin or an underlying matrix.

Previously KAP1 phosphorylation was shown to be dependent on ATM signalling [7], but other studies [8] show that KAP1 phosphorylation occurs in ATM deficient cells, demonstrating that KAP1 might be redundantly targeted for phosphorylation by all three nu-