Chromatin dynamics during DNA repair investigated *via* chromatin-directed proteomics

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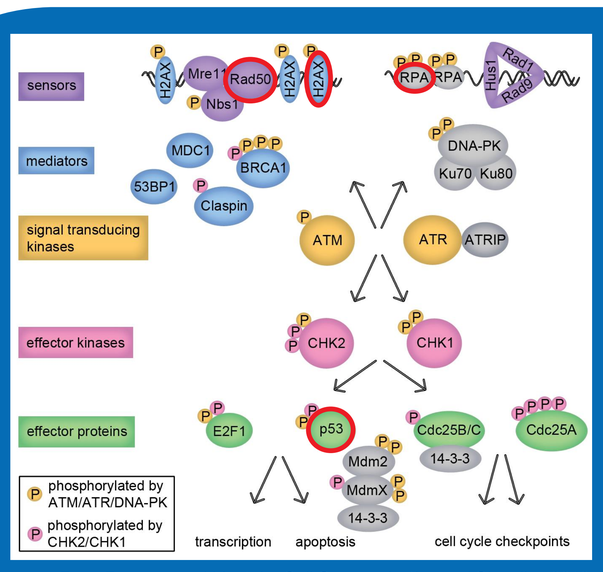


From Freeman and Monteiro Cell Communication and Signaling 2010

Abstract

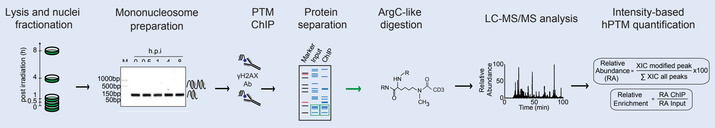
DNA lesions predispose to genomic instability, a hallmark of cancer; therefore cells have evolved repair pathways to solve those harmful insults. Double-strand breaks (DSBs) represent the most lethal DNA damage first marked by the phosphorylation of the histone H2A.X (γH2A.X) which triggers the recruitment of sensor proteins belonging to either the error-prone non-homologous end joining (NHEJ) or the efficient homologous recombination (HR) pathway. It is now established that chromatin has an active role also in DNA repair, thus its characterization at DSB repair foci is essential to better understand the coordinate action of the repair mechanisms and to identify novel players participating in tumor-associated apoptotic resistance and cell survival.

Here we dissect chromatin changes upon exposure to ionizing radiations through multiple proteomics-based approaches. We applied the Selective Isolation of Chromatin-Associated Protein strategy (ChIP-SICAP; Rafiee, 2016) to investigate the interactors of core NHEJ, HR proteins and γH2A.X while bound to the DNA or in the chromatin soluble fraction. Through a click chemistry-assisted procedure we profiled the configuration of DNA-bound proteins during DSBs repair; finally we analyzed the histone post-translational modifications (hPTMs) cross-talk at mono-nucleosomes marked by γH2A.X.

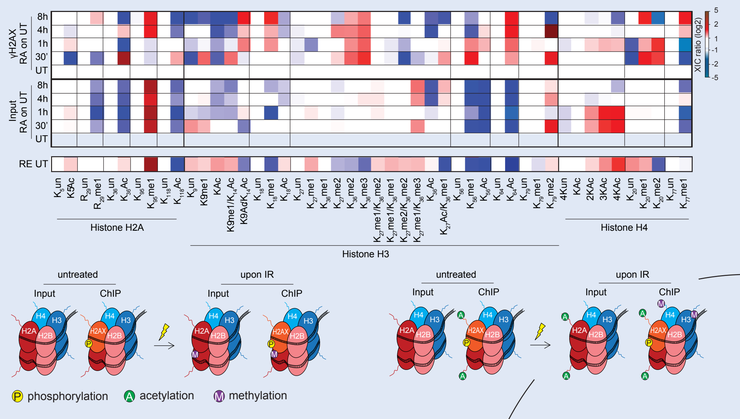
Our integrated analysis identified the dynamics of expected chromatin determinants during the DNA repair and interestingly suggested the role for new candidates specifically enriched upon DSB formation. Validation experiments based on monitoring of DSB foci formation and resolution in AID-DIvA cells proficient or knock-down cells provided evidence of a role for novel candidates in DNA repair. FACS-based analysis of Traffic-light Reporter (TLR) isogenic cells upon silencing of proteins identified by MS characterized their functional role in NHEJ, HR or pathway choice. Furthermore, we defined hPTMs associated with γH2A.X-marked mono-nucleosomes and their dynamics during DSB resolution. This analysis corroborated expected enrichments (e.g. H4K20me1/me2) and provided insights on new modifications specifically enriched at γH2A.X-nucleosomes.

1. hPTMs cross-talk during DNA damage

A) Time-course hPTMs profiling upon IR at mono-nucleosome resolution

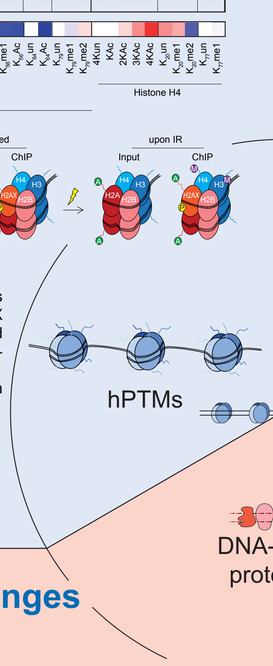


B) Two different scenarios for hPTMs associated with γH2AX



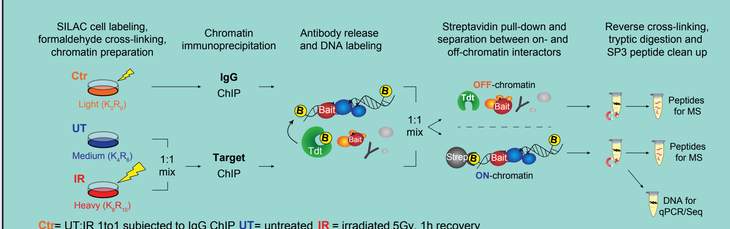
A) Native preparation of mono-nucleosomes from untreated as well as cells during the DNA damage response was used as input for γH2AX ChIP followed by Native chromatin proteomics (N-ChroP[2]) MS-based identification of co-associated histone post-translational modifications.

B) The relative abundance of histone PTMs were profiled over time in input as well as in γH2AX-enriched mono-nucleosomes.
Two scenarios were identified:
- modifications showing similar dynamics in both γH2AX-containing mono-nucleosomes (local) and in input chromatin (global).
- hPTMs specifically associated with γH2AX nucleosome (e.g. H4K20me1/me2, H3K56me1. H3K64Ac).



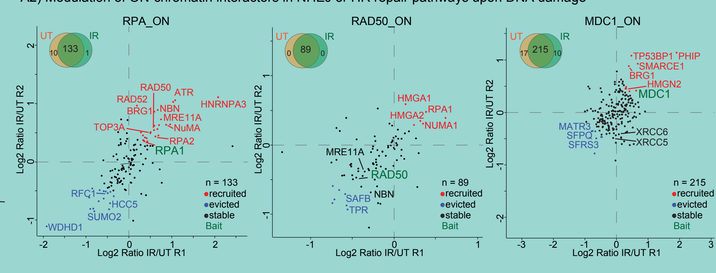
2. Functional ON-chromatin interactors of NHEJ, HR and γH2AX identified by SICAP

A) Selective Isolation of Chromatin-Associated proteins in NHEJ and HR

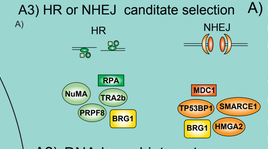


Ctr= UT:IR 1to1 subjected to IgG ChIP UT= untreated IR = irradiated 5Gy, 1h recovery

A2) Modulation of ON-chromatin interactors in NHEJ or HR repair pathways upon DNA damage



A3) HR or NHEJ canditate selection

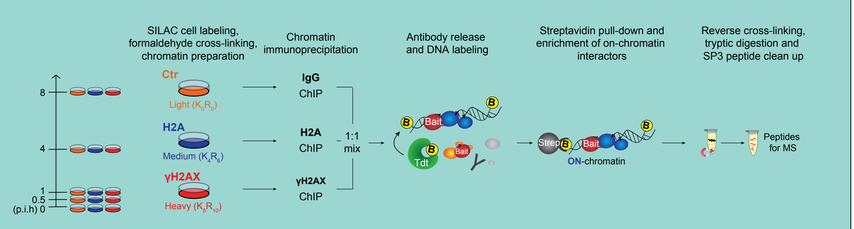


A) ON-chromatin interactors of core components of the MRN (RAD50) complex, HR (RPA) or NHEJ (MDC1, TP53BP1) DSB repair pathways were characterized by an optimized ChIP-SICAP protein in untreated and cell exposed to sub-lethal IR doses.

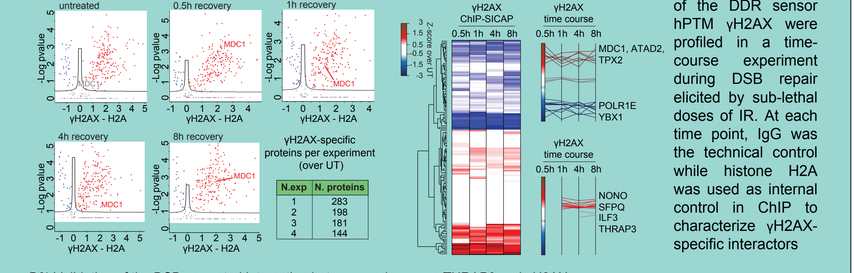
A2) DNA-bound interactors were quantified by means of SILAC protein labeling and chromatin determinants were classified into recruited (red), evicted (blue) or proteins stably associated (black) with RPA, RAD50 or MDC1. Based on these results, it seems that the MRN complex interacts more closely with HR rather than NHEJ, where a higher number of chromatin regulators were identified.

A3) Through integrated analysis, some interesting candidates specific for either HR or NHEJ were selected for further functional validation experiments (see box.4).

B) Dynamic profiling of γH2AX-associated proteins during DSBs repair



B2) Identification of γH2AX-specific interactors and their dynamic characterization upon DNA damage

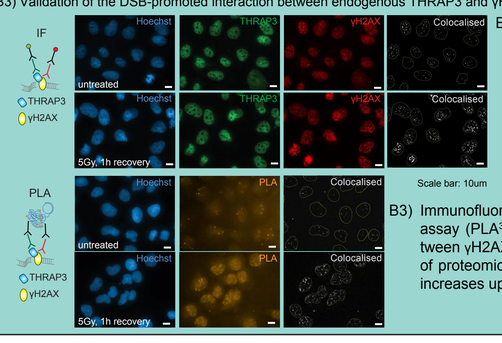


γH2AX-specific proteins per experiment (over UT)

N.exp	N. proteins
1	283
2	198
3	181
4	144

B) Chromatin interactors of the DDR sensor hPTM γH2AX were profiled in a time-course experiment during DSB repair elicited by sub-lethal doses of IR. At each time point, IgG was the technical control while histone H2A was used as internal control in ChIP to characterize γH2AX-specific interactors

B3) Validation of the DSB-promoted interaction between endogenous THRAP3 and γH2AX

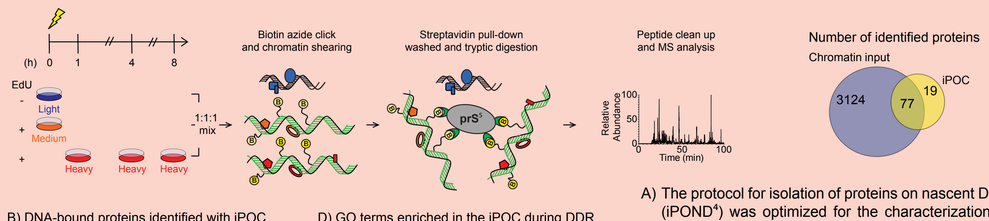


B2) Volcano plots show the fold-change difference between γH2AX and H2A, MDC1 is depicted as positive control. γH2AX-specific interactors identified at all the time points are reported in the heat-map and the corresponding line plots show the time-course behaviour of those interactors. Of interest, the protein THRAP3 is enriched at γH2AX in particular after 1h upon IR.

B3) Immunofluorescence (IF, top) and proximity-ligation assay (PLA[3], bottom) validation of the interaction between γH2AX and endogenous THRAP3. As validation of proteomic results, the number of interactions spots increases upon IR-mediated DNA damage.

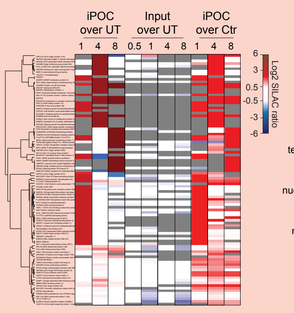
3. DNA-bound proteome changes during DSB repair

A) isolation of Proteins On Chromatin (iPOC) during DNA repair in comparison with chromatin input

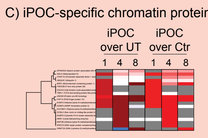


A) The protocol for isolation of proteins on nascent DNA (iPOND[4]) was optimized for the characterization of the chromatin composition in terms of proteins stably bound to the DNA (iPOC). EdU treated (or not as negative control) cells were labeled in SILAC and the heavy channel corresponds to similar time points upon IR as in box 1 and 2B. Upon on-beads tryptic digestion (on protease-resistant beads, or prS[5]) DNA-bound proteins were identified and compared with determinants identified in in-depth chromatin input

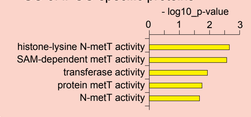
B) DNA-bound proteins identified with iPOC



C) iPOC-specific chromatin proteins



GO of iPOC-specific proteins



D) GO terms enriched in the iPOC during DDR

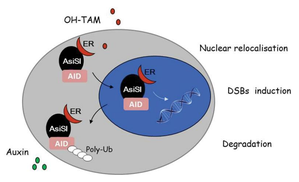
B) Proteins identified in iPOC are reported in the heat-map. Strikingly, the majority of the determinants show a modulation over the untreated sample only in iPOC while in the input, if identified, don't change. All the reported proteins are enriched over the techinical control (- EdU).

C) Subset of determinants quantified only in the iPOC experiment. All these proteins are highly enriched over the untreated sample at at least one time point, thus representing proteins specifically recruited at DNA upon DSBs elicited by sub-lethal doses of IR. Gene ontology (GO) analysis of these deteriminats shows a over-representation for proteins involved in methyl-transferase activity to histone lysine residues. Among those, of particular interest are methylations on H3K4 and H3K9 enriched in mono-nucleosomes marked by γH2AX (see box.1)

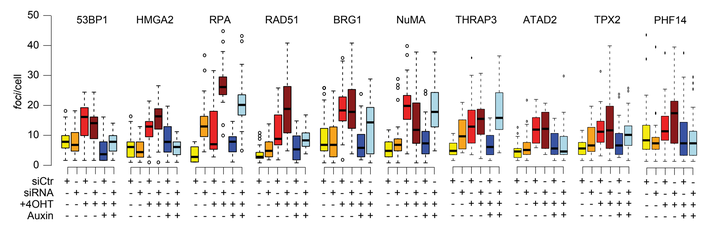
D) GO classification of proteins enriched in iPOC at 1h (green), 4h (orange) or 8h (red) upon IR-induced DSB. From these results, we can speculate that DNA repair proteins are recruited at DNA within 1h from DSB, followed by determinants involved in gene expression regulation (at 4h) and restoration of the chromatin structure upon damage repair (8h)

4. Functional validation with site-specific DSBs cellular systems

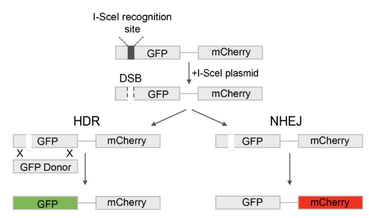
A) Auxin-Inducible Degron DSBs Induced *via* AsiSI (AID-DIvA[6]) cells



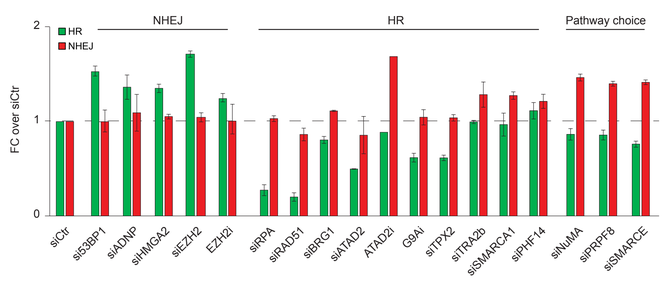
Validation of NHEJ-, HR- or γH2AX-associated candidates



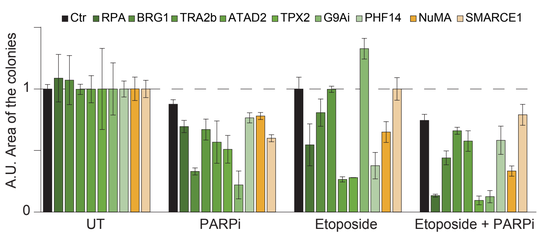
B) Traffic light reporter assay[7,8]



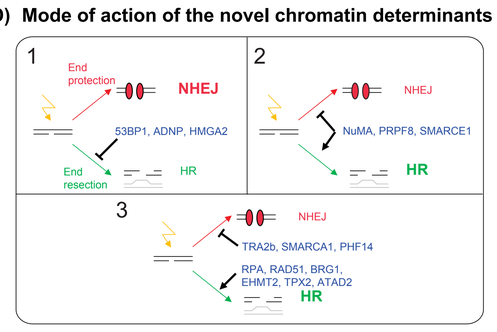
TLR assay normalised on siRNA Ctr



C) Colony formation assay to screen for synthetic lethality



D) Mode of action of the novel chromatin determinants



Conclusion and remarks

- At mono-nucleosome resolution the analysis of γH2AX-associated hPTMs suggests local (DDR mono-nucleosomes) cross-talks and dynamics compared to global chromatin (input).
- The integrated analysis between chromatin-associated proteins specifically characterize the functional interactors of either HR or NHEJ core components while on the DNA.
- The time course ChIP-SICAP experiment of γH2AX identifies the time-dependent dynamics of determinants upon IR and shed light on novel targets in DSB repair e.g. THRAP3
- DNA-bound proteome (iPOC) outperforms deep chromatin proteome by profiling both determinants with functional role in DSB repair pathways and chromatin modifiers.
- Orthogonal functional validation determines the role in DDR for proteomic candidates and identifies novel players of HR repair, thus opening therapeutic opportunities with PARPi.

***References**: 1 Rafiee Mol Cell, 2016; 2 Soldi MCP 2013; 3 Soederberg Nat.Meth. 2006; 4 Sirbu Gen&Dev 2011; 5 Rafiee Sigismondo Mol Syst Biol 2020, 6 Aymard NSMB 2014; 7 Certo Nat.Meth. 2011; 8 Abu-Zhayia SciRep 2017*