Does Deleting the Protein Cohesin Change DNA Associated Modifications in Cell Division?

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AIMS

Based on the observation that CTCF & cohesin colocalise with the kinase Haspin, the aim of this project was to analyse the phosphorylation status of H3T3 after depletion of either the cohesion subunit SCC1 or the transcriptional regulator CTCF.

INTRODUCTION

Haspin is a mitotic enzyme which has a role in the protection of centromeric cohesion and phosphorylates (ph) threonine at position 3 on Histone 3 (H3T3ph), a protein required to organise DNA[(1)(2)]. This ph recruits the chromosomal passenger complex for accurate segregation of replicated chromosomes which is vital to cell division[(1)(2)]. However, the role of H3T3ph in other regions is not yet known.

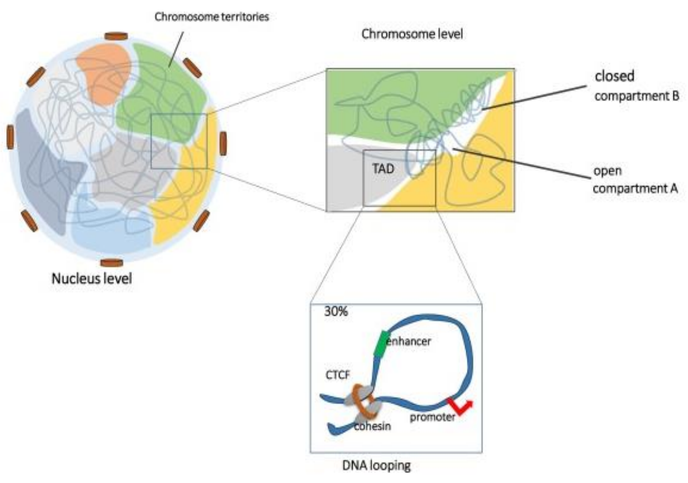


Figure 1: Shows the structure and formation of TADs[(3)]

CTCF binds to the DNA and along with cohesin forms structures called TADs (Topologically associating domains) (Figure 1). TADs are clusters of loops in the DNA, and they function to bring distant DNA elements together to regulate gene expression[(3)]. However, TADs are lost during mitosis.

ChIP-seq was used to show that CTCF and H3T3ph are found in the same regions but at different stages of the cell cycle. This relationship suggests that H3T3ph could bookmark the position of TADs before mitotic entry.

METHOD

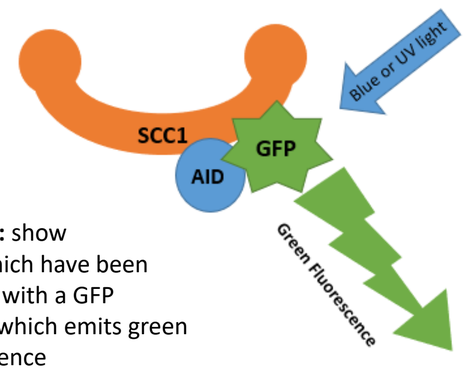


Figure 3: show SCC1 which have been labelled with a GFP protein which emits green fluorescence

Live

CTCF or SCC1 proteins were labelled with green fluorescent protein (GFP) and an auxin-inducible degron (AID) to make two HeLa cell lines (SCC1-AID and CTCF-AID). Addition of auxin then targets the proteins for degradation in these cells. This was visualised by live fluorescent microscopy. Images were recorded at 1 min intervals for 30 min from which the intensity of GFP was measured using ImageJ software.

Chromosome Spreads

SCC1-AID cells were treated with nocodazole to arrest in mitosis and then either treated with auxin for 24 hour or left untreated. For both samples, mitotic cells were physically shaken free for collection and swelling solution was added. Cells were then spread on microscope slides. The H3T3ph and ACA were visualised by immunofluorescence (IF).

Immunofluorescence

IF (Figure 4) was used to visual specific antigens in the SCC1-AID cells including ACA (centromeric marker) and H3T3ph. The Antibodies (Ab) were added to fixed control cells or cells treated with auxin for 24 hours. Therefore we could see what effect the loss CTCF and SCC1 have on these antigens. DAPI was also used to stain the DNA so that it could be visualised.

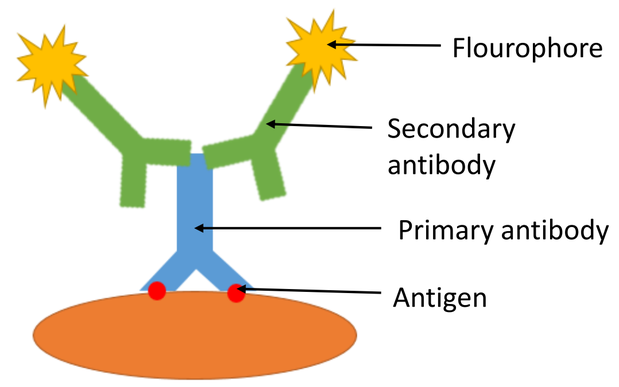
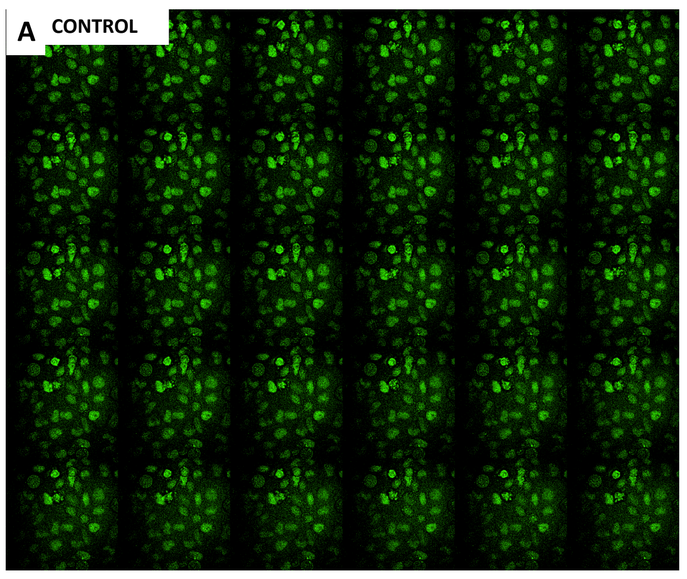
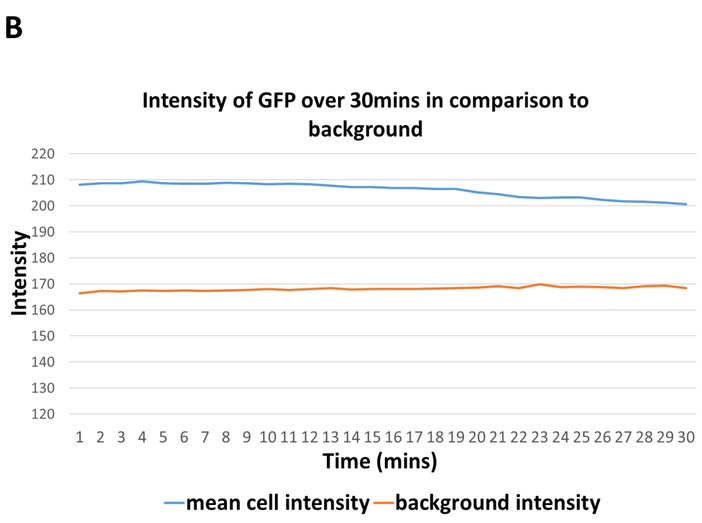
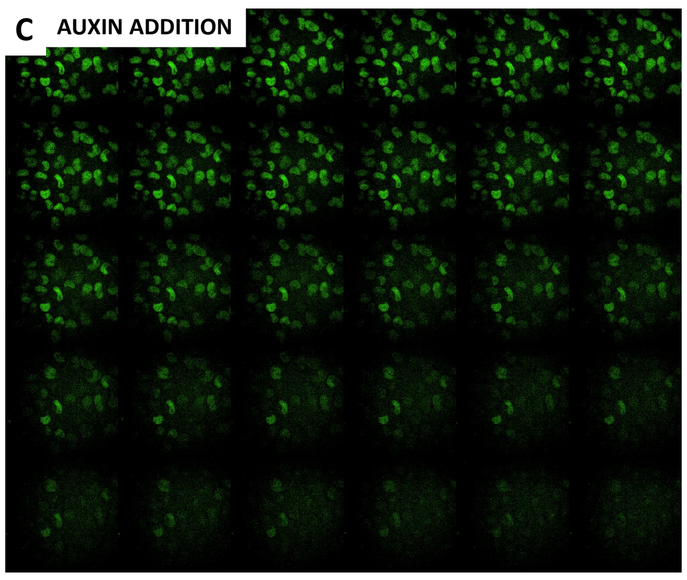


Figure 4: IF uses two antibodies (Ab) to image a specific antigen. Primary Ab binds to the antigen and secondary Ab binds to primary Ab to amplify the signal.

RESULTS







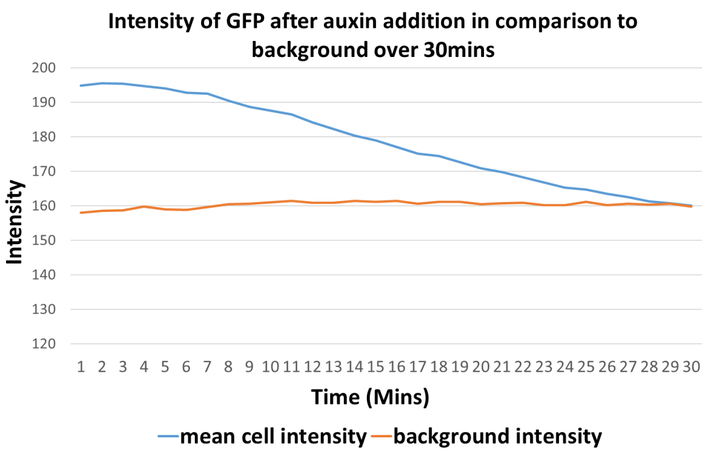
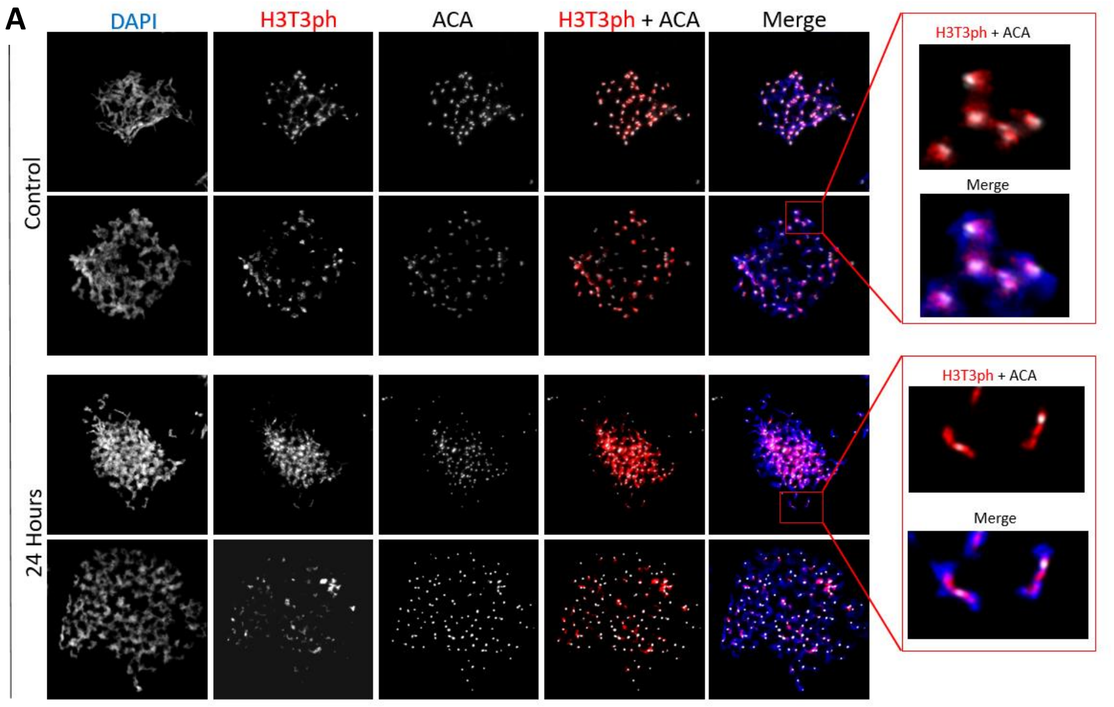


Figure 5: A) Images taken at 1 min time points over 30 min observation period. **B**) Shows the intensity of GFP in control cells at indicated time points in comparison to background intensity **C**) Images of cells treat with auxin at 1 min time points for 30 min following auxin addition **D**) Intensity of GFP in the cells at these time points in comparison to background intensity (the same was seem for CTCF but not pictured)



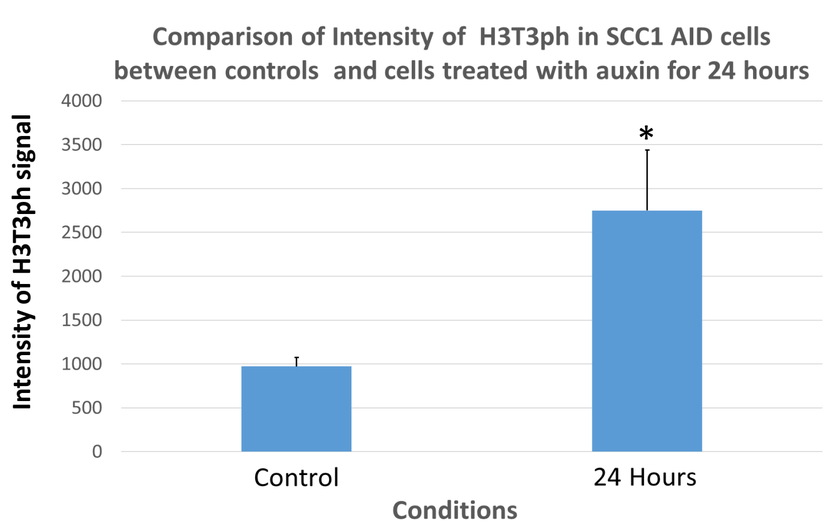


Figure 6: A) SCC1 Cells in different conditions where the chromosome have been spread and imaged by IF **B**) Highlights the intensity of H3T3ph staining on the arms of chromosomes in SCC1 AID cells in different conditions (t-test * $p<0.05$, n=15)

DISCUSSION

When auxin is added to both SCC1-AID and CTCF-AID Hela cells there is a rapid degradation (as measured by loss of GFP fluorescence) of either SCC1 or CTCF respectively with almost complete degradation by 30 min (Figure 5C,D), with degradation commencing at around the 5-7 min (Figure 5D), and in complete contrast to the control (Figure 5A,B). When SCC1 was lost from the cell, cohesion was compromised as expected (sister chromatids, normally bound together by cohesin, were separated; Figure 6A). To analyse how localisation and levels of H3T3ph changed with the loss of SCC1, chromosomes in mitotic SCC1-AID cells were subject to spreading (Figure 6). H3T3ph signals were located at the centromeres in controls whereas, after auxin addition, it occurs more on the arms (Figure 6A). Indeed, by comparing the intensity on the arms between conditions, we found that cells treated with auxin had a significantly increased H3T3ph signal on the arms (Figure 6B). Preliminary experiments suggest that CTCF does not show as profound an affect on H3T3ph.

Conclusion: The cohesin subunit SCC1 is required for the normal location and intensity of H3T3ph at centromeres in mitosis. Further work is required to determine is this affect is specifically due to the loss of SCC1 or due to the disruption of cohesion which happens when SCC1 is lost.

References: 1. Wang F, Ulyanova NP, van der Waal MS, Patnaik D, Lens SM, Higgins JM. A positive feedback loop involving Haspin and Aurora B promotes CPC accumulation at centromeres in mitosis. Current Biology 2011;21(12):1061–1069. **2**. Zhou L, Liang C, Chen Q, Zhang Z, Zhang B, Yan H, Qi F, Zhang M, Yi Q, Guan Y, Xiang X, Zhang X, Ye S, Wang F The N-Terminal Non-Kinase-Domain-Mediated Binding of Haspin to Pds5B Protects Centromeric Cohesion in Mitosis. Current Biology 2017;27(7):P992-1004 **3**. Wang DC., Wang W., Zhang L., Wang X. A tour of 3D genome with a focus on CTCF. Seminars in Cell & Developmental Biology 2019;90:4-11