The Spatio-Temporal Interaction Dynamics and Localization of the Anaphase Promoting Complex (APC/C) and its Substrate Cyclin B1 in Human HeLa cells

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Introduction

The anaphase-promoting complex or cyclosome (APC/C) is assembled from atleast 13 different proteins, it is an unusually large multi-subunit cullin-RING E3 ubiquitin ligase [1]. Its function in the cell cycle is to control sister chromatid segregation and the exit from mitosis into the S-Phase by catalysing the ubiquitylation of cell cycle regulatory proteins mainly securin and cyclin B1 for destruction. Importantly, APC/C-Cdc20 activity towards securin and cyclin B is inhibited by the mitotic checkpoint complex (MCC), a multi-protein complex generated in response to the spindle assembly checkpoint (SAC) [3] The mitotic checkpoint complex (MCC) formed by Mad2, BubR1, Bub3 and Cdc20 is an inhibitor of APC/C. This allows faithful and accurate separation of sister chromatids during mitosis.

The APC/C spatial-temporal profiles of substrate ubiquitination, as well as substrate interaction with Cdc20, are not yet known. Therefore we are aiming to address this in this study.

AIMS

Our main objective was to study the formation profile of mitotic checkpoint complex (MCC) in HeLa cells treated or none treated with Nocodazole and Taxol by performing in vivo protein-protein interaction antibody staining with antibodies against the components of the MCC. To study the Spatial-Temporal Profile of the Interaction between Cdc20 and Cyclin B.

Methods

Method 1: Cell culture, establish, maintain and synchronisation of HeLa cells

HeLa cells were maintained in complete DMEM with 10% FCS, 1% Pen-Strep, 1% Glutamine. For synchronization a double thymidine block was carried out, cells were treated with 2mM Thymidine. This blocks 90% of the cells in early S-phase To block HeLa cells in prometaphase cells were treated with nocodazole or taxol after the syncronisation.

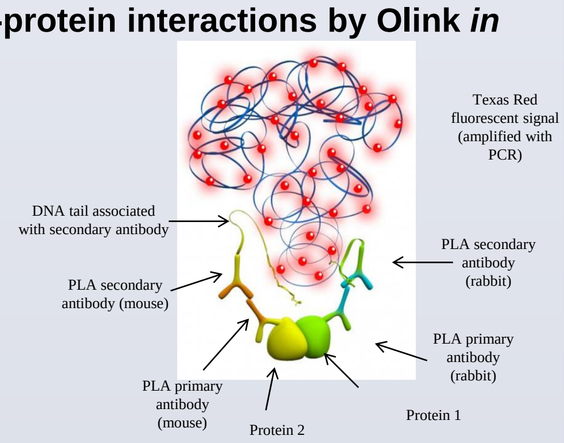
Method 2: Visualisation of protein-protein interactions by Olink *in situ* PLA Staining Technology

Figure 2. This study used *in situ* ligation assay (PLA). PLA utilizes a fluorescproximity ence based analytical approach using two primary antibodies raised in different species to recognize the target antigen(s) of interest [2].

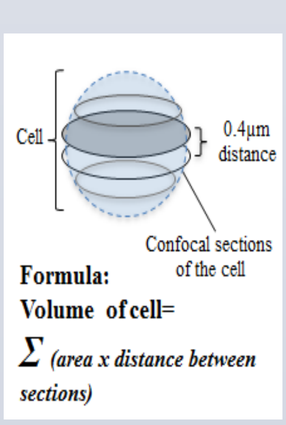
Two species-specific PLA secondary antibodies, each containing a short DNA sequence that can be used for signal amplification by PCR after ligation.

The technique can detect protein-protein interaction at a proximity of less than 40nm.

The fluorescent signals are detected using confocal microscopy with appropriate excitation wavelengths. (Figure adapted from Ref. 2)



Method 3: Visualization of complex fluorescent intensity using maximum projection of Z-stack confocal images



Cdc20-Cyclin B1

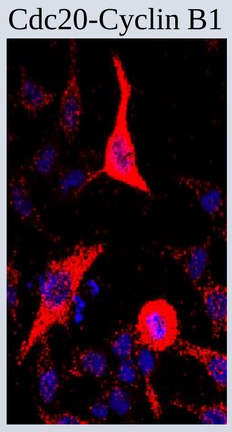


Figure 3. Example of maximum projection confocal images of a Z-stacks, showing interaction between Cdc20 and Cyclin B, used for measuring the maximum fluorescent intensity. The DNA (DAPI, 405nm) is shown in blue; the protein complexes are shown in red (Texas Red, 594nm).

Leica TCS SP2 confocal laser-scanning confocal microscope was used, with a 40x oil objective lens and consistent power settings.

Method 4: Measurement of Fluorescence Intensity Using Image J Software

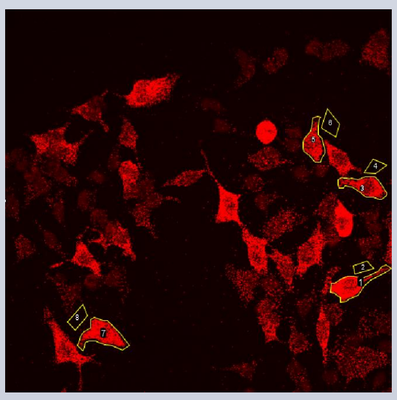


Figure 4. The regions of interest (ROIs) were defined and the fluorescence intensity (FL) at different cell cycle stages were quantified using Image J software as indicated.

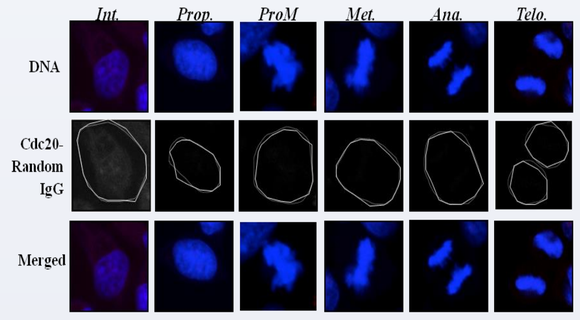
#1-8 are ROIs
#1, 3, 5 and 7 are cells of interest.
#2, 4, 6 and 8 : The ROIs used as background (bg) FL for subtraction

The Total FL= Integrated density of cell –[(Bg integrated density / Selected Bg Area) x Cell Area]

Results and Conclusions

Result 1: Control Primary Antibodies Specificities Test Using Random IgG

Figure 5. The specificity of the primary antibodies used in this study (with the exception of anti- ubiquitin antibody) have been tested by use of an appropriate random IgG as the negative control. The figure revealing no detectable signal were observed when Cdc20 is paired with a random IgG as an example. This suggests that the fluorescent signals obtained in this study are representative of protein-protein interaction of the chosen pairs of the study.



Result 2: A selected example of Cdc20-Cyclin B1 (CycB) interaction throughout the cell cycle stages

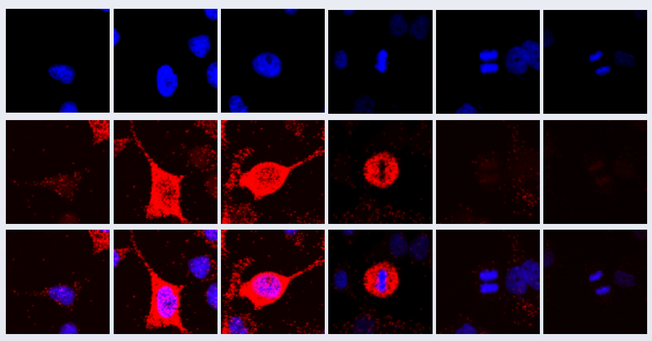


Figure 6. Cdc20 and Cyclin B1(CycB) interaction can be detected in interphase. This interaction rapidly increases and reaches a peak at prophase and prometaphase. Levels are significantly reduced in metaphase. It is no longer detectable in anaphase and telophase cells.

Result 3: Comparison the interaction patterns between CycB-Ub, Apc3-Apc6, Apc6-BubR1 and the distribution of the CycB at cell cycle stages

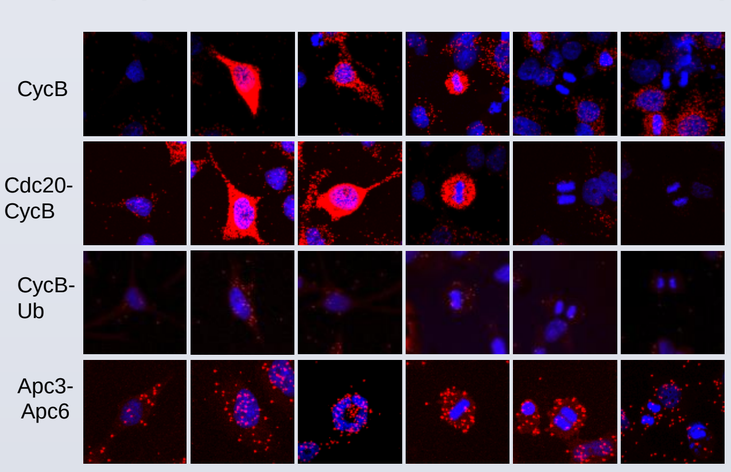


Figure 7. CycB protein levels sharply increase and reach a peak at prophase. Levels begin to decline at prometaphase and metaphase. It is no longer detectable at anaphase and telophase.

The interaction patterns of Cdc20-CycB and CycB-Ub (ubiquitin) are rather similar to the distribution pattern of CycB, although lower levels of the CycB-Ub were detected.

The levels of APC/C are observed to remain relatively constant throughout the cell cycle.

Result 4: Comparing the levels of complex of the CycB and the interactions between the Cdc20-cycB, CycB-ub, and Apc3-Apc6 at different cell cycle stages

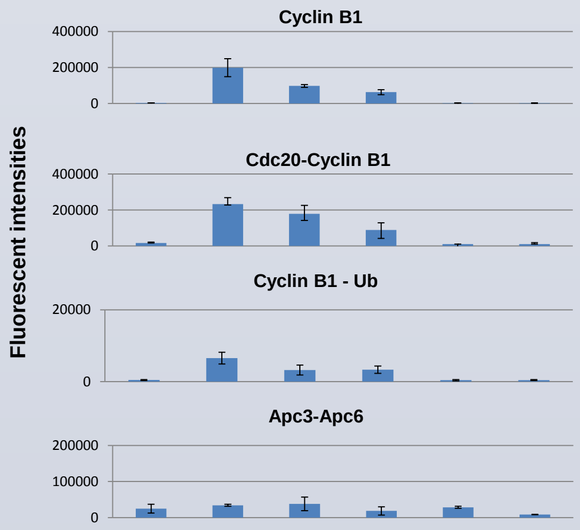


Figure 8. Using quantification to analyse the results the graphs in figure 8 shows different stages of the cell cycle and the relative amount of complex. The of CycB and the patterns of interaction between the Cdc20 and CycB, as well as between CycB and Ubiquitin, are consistent with the observation from the results indicated in results 3. This suggest that APC/C exists throughout the cell cycle and remains at relatively constant levels at all stages as indicated.

Acknowledgements

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References

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